

## The End of Rowland Hill

THE strike of the British Post Office workers, now mercifully ended, will probably become a classic example of discontinuous industrial change. Two months ago, before the strike began, it was clear that the British mail services were declining slowly yet gracefully. Since the mid-sixties, indeed, the volume of letters and parcels handled has been shrinking, no doubt because of higher charges but also because there has been increasingly fierce competition from other methods of communication, especially the telephone. What has happened now is that the drift away from the mails has been accelerating. For one thing, many organizations have discovered that there are often cheaper ways of delivering pieces of paper in urban communities than the services provided by the Post Office. And it will be interesting to see how permanent is the loss of business to the Post Office Corporation on this account. In the long run, however, the most lasting—and for the Post Office the most damaging—consequence of the strike will be the way in which it has stimulated the use of telecommunications. Not merely have people learned new habits and discovered hidden advantages of telephones, telex terminals and the like, but the chances are that the Post Office Corporation will be able to balance its books only by increasing still further the charges made for carrying letters about the place, with the result that the economic advantage of telecommunications will be still further enhanced. For the Post Office Corporation as well as for the British government, this development can be regarded either as a disaster or as an opportunity. In short, this is a time when both parties must look for policies that make the best of a new situation.

Where the mail services are concerned, the urgent need is to acknowledge which way the wind is blowing. There is no way to halt the flight of business from the mail services. In strictly commercial terms, the Post Office should be encouraged or even compelled to change its pricing policy in such a way as to reflect more accurately the cost of the actual services which it provides. Thus there is a strong case for letting the charges made for handling letters include something extra where hand delivery is required or where items of mail originate from or are consigned to rural areas in which the costs of handling are known to be very large. So much, indeed, has been desirable for several years. In 1967, the Select Committee on the Nationalized Industries pointed out that the average cost of handling a letter posted in the country was something like 25 per cent greater than the national average, and that the same economic penalty applied to the delivery of letters. Moreover, it also emerged from the same public inquiry that for a substantial amount of mail, the real handling costs are several times the charges levied. So is there not a case for charging extra for rural mail? In 1967, both the British Treasury and the Post Office argued against such an expedient chiefly on the grounds that "not only country dwellers benefit from the services provided in rural areas; their correspondents in towns also benefit". This, of course, is the old but enlightened doctrine of

Rowland Hill. The difficulty is that in present circumstances the Post Office Corporation can only regard this ideal as the principle of its operations by driving away the customers in the cities who at present generate the profitable part of the Post Office's mail business. In the long run, the result could easily be that country correspondents would be more severely deprived than if they were at present required to pay a more realistic price for the service they receive.

Without much difficulty, the Post Office could and should go much further along this same road of making its charges reflect the true costs. As things are, the charge for posting a letter is independent of whether it will have to be delivered by itself or in company with a great number of other letters, yet evidently the first case is more expensive for the Post Office than the second. Should there not, in other words, be some kind of rebate for bulk delivery or, more workably, a charge for single delivery which is mitigated by bulk delivery? There is also a case for looking again at the old Rowland Hill notion that postal rates should be independent of distance travelled. In the days when long distance transport was comparatively cheap, and when the Post Office's costs consisted largely of the cost of employing people to sort and deliver letters, it might have been fair to suppose that transport between distant towns was unimportant. In present circumstances, and especially if tariffs reflect more accurately the cost of delivery, it could easily be that long distance transport costs will be an important factor. But this may in due course imply that there may be good sense in some arrangement for letting postal charges reflect the distance travelled. The notion that the British Post Office should be divided into several independently accounting regions is not to be dismissed.

So has the time come to abandon the principle that communication should be equally easy and inexpensive between all parts of a country the size of Britain? As good luck will have it, as the mails are pricing themselves out of the long distance business, the telephone service is better placed than ever to provide a substantially uniform tariff. Increasingly, the cost of trunk lines is a diminishing part of the capital cost of the telephone service—that is one of the benefits of technology in the past few years. Indeed, as automatic methods of working spread throughout the telephone system, it is more and more likely that there could be a tendency towards a tariff structure in which the fixed cost per year of being connected to the system became more important to the user than the charges levied for individual telephone calls, local or long distance. And certainly there is no reason why the Post Office Corporation should not steadily work towards a state of affairs in which trunk calls—even international trunk calls—are hardly more expensive than local calls. That is the modern equivalent of the uniformly priced communications service on which the Victorians set their hearts. The fact that it seems now more within reach than ever is yet another reason for regarding the postmen's strike as an irrelevance.



The question is merely whether the Post Office operation will be able to meet the demand for more and better services now likely to be made on it. The years ahead may be full of opportunities, but the past decade has been a great misfortune for the development of an efficient telecommunications network. The old Post Office (before it was set up as a public corporation outside the civil service) was a strange mixture of conservatism and foolhardiness. Some of the biggest mistakes were those in which the Post Office set out to develop radically new equipment with inadequate resources and without a proper conception of how it could be used—an abortive attempt a decade ago to use a computer as an electronic telephone exchange was foolhardy rather than conservative. But elsewhere the Post Office has stuck to

old-fashioned equipment for switching circuits when other telecommunications authorities have been installing telephone exchanges of a more efficient and flexible design. The result is that by the late seventies, the British Post Office will be the largest user of Strowger switches in the world. Its hope is that by the end of the decade, it will also be forging rapidly ahead with the installation of new equipment built around the digital transmission circuits now being installed and the digital switching exchanges that will eventually connect them. The biggest danger is that, once again, the Post Office's hopes will have leapt ahead of the budget it can look forward to. Certainly the capital investment needed to transform the present system into one capable of meeting the objectives that now seem desirable will be large by any standards

## Where Doctors Draw the Line

THE British medical profession may find itself embarrassed by the outcome of the hearing before the General Medical Council last week of the case of Dr Robert Browne, who was accused by a birth control clinic in Birmingham of disclosing to the parents of a teenage girl that the clinic had been supplying the girl with contraceptive pills. On the face of things, telling the parents was a breach of confidence and so, indeed, the General Medical Council considered it to be. But the council went on to say that the disclosure of this information had not been considered to be improper, and that the doctor was therefore not guilty of "serious professional misconduct" as the clinic had urged. The difficulty of the case is not so much that it may in future make life hard for the birth control clinics—the climate of opinion is now such that general practitioners are increasingly accustomed to help out the clinics—but that the decision of the General Medical Council will further complicate the relationship between doctors and their patients, especially those who cannot easily answer back. The verdict also raises important and difficult questions about the way in which society must regard the transition from childhood to adulthood, theoretically complete (since Parliament pronounced on the subject last year) at the age of eighteen.

Doctors are inevitably subjected to a great many painful questions like that which Dr Browne seems to have felt compelled to answer. It is in the nature of their business, for example, that they should learn without asking a great deal of confidential information about their patients who are, again in the nature of things, very often personal friends as well as patients. Thus doctors are very often drawn into discussions of marital mix-ups. They learn from insurance companies and even from patients themselves a great deal about the personal life of people who would often be aghast at the thought that this should be more widely disseminated. Most doctors are well aware of the nature of these confidences and thoroughly skilled at keeping them. It is also only fair to say that most doctors seem sensitively aware of when it is necessary to break a confidence as, for example, when it is necessary to tell a close relative of some fatal illness in a patient. To be sure, in the case of the sixteen year old girl and her family, the outcome seems to have been nothing other than desirable—obviously it is better for the girl and for her parents

that both sides should know what is going on.

In other circumstances, even Dr Browne's breach of confidence could have had more serious consequences. What if an angry parent had shown his wayward daughter the door? What if some other Victorian melodrama had been triggered off by his indiscretion? It is only partly an answer to say that the doctor knew the family sufficiently well to know how they would respond. For one thing, doctors are not necessarily good at this kind of prognosis. For another, there is no assurance that all the doctors who will now regard the General Medical Council's verdict as a precedent to follow will be as discriminating or as judicious. In short, the decision gives general practitioners too much licence. The proper course for Dr Browne to have followed would have been the course which doctors should always seek to follow if they think they must interfere in family affairs—to persuade the patient most directly concerned to make the disclosure himself. In short, what Dr Browne should have tried was to persuade his teenage patient that she should have told her parents that she took the Pill. If that attempt had failed he would have been on much more sure ground in telling her parents.

But what rights have children and teenagers in matters like these? If the case against Dr Browne had been that he had informed a husband that his wife was taking contraceptive pills, and if this were an issue between the pair, the result of the General Medical Council's inquiry would undoubtedly have been quite different. Only because his patient on this occasion was a minor was the breach of confidence considered to be innocuous. Yet there can be no simple dividing line between minority and adulthood. If anything, the need of physicians to win the confidence of younger patients should help somehow to ensure that doctors are more forthcoming than, say, policemen in their readiness to treat teenagers as adults. For most of them, this is the best way to ensure that teenage patients will talk freely about precisely the kinds of problems that gave offence to Dr Browne. The result, in other words, of his over-hasty action could easily be to persuade other teenagers than his patient to keep confidences to themselves. How many teenage drug users will now, for example, be more reluctant than in the past to seek advice from their doctors or even from drug treatment centres for fear that the news will be passed on to other parties not directly concerned?



## General Motors Beware

HARVARD University will be the envy of a great many other similar institutions for the way in which it can bare its breast in public on the policy to be pursued on financial investments in commercial corporations (see page 78). Few other institutions, after all, are sufficiently well endowed for the responsibility to lie as heavily on them as it does on Harvard. But Harvard is also now in a mood for public soul searching of a delicacy and dedication only to be matched by those occasions in the recent past when the University of Oxford set out to decide whether there should be a course in human biology.

The trouble with the Austin committee's report on the management of investment policy is that there can at this stage be no assurance that the process of consultation will be workable. The chief proposal is that there should be a university officer separate from the treasurer who would collect opinions about the non-financial aspects of corporations with stocks in the university's investment portfolio, and who would suggest to the corporation of the university how the strict rule that financial returns should be maximized might be modified by non-financial considerations. The difficulty, of course, is that there are very few circumstances in which these non-financial considerations are clear-cut. The committee is safe enough when it suggests that corporations which practise racial discrimination should be fought against,

first of all by not investing new money in their stock and then by seeking to use the stockholders' power to change the way the corporation behaves, but racial discrimination is in any case illegal in the United States. But what about Women's Liberation? Cannot the luckless holder of the office which the Committee on University Relations with Corporate Enterprise would create expect to find his first few days consisting of an attempt to outlaw from Harvard's investment portfolio all the corporations which do not offer equal pay for women? And although the Austin committee is judiciously careful not to say what it thinks are the rights and wrongs of the case against General Motors, are not there likely to be vigorous demands that all corporations which contribute to pollution—atmospheric (motor manufacturers), noise (Boeing), water (Dow) and cultural (CBS)—should be robbed of Harvard capital? If the holder of the new office enjoyed his job, and were prepared to regard himself as a conducting rod for all the conflicts with which universities are likely to be concerned, he might perform a valuable service. But if, as seems most probable, he will seek to avoid trouble by giving advice to sell investments before the pressure has built up, the result will be to erode the principle that the university's finances should be maximized and, at the same time, to avoid debate. That could easily be the worst of both worlds.

## UNESCO Cuts Its Throat

It now seems clear that the General Assembly of UNESCO has made a simple but easy error in deciding last year that the organization should in future have no dealings with non-governmental organizations which themselves have refrained from severing diplomatic relations with South Africa. As with cricket, so with culture, it seemed at the time quite proper to make some gesture of protest at the policy of *apartheid* to which the government of South Africa is devoted. The argument which seems to have carried weight with those of the UNESCO delegations not voting automatically was that cultural isolation would bring the government of South Africa in due course to mend its ways. The snag, of course, is that the organizations which are not governments which nevertheless rely on UNESCO for support of various kinds include entirely worthy bodies such as the International Council of Scientific Unions, within which it is quite unthinkable that there should be any political discrimination. If, after all, Davy and Faraday could have gone jaunting around the mainland of Europe during the Napoleonic Wars, is it sensible that their intellectual successors should cut off their relationships with scientists in South Africa because the government of South Africa pursues racial policies which are offensive not merely to outsiders but often to many of those on the spot as well? And is it credible that such a step would do more than confirm the Nationalist Government in its wayward course? And if there is within the framework of an organization supporting ICSU and other good works a convention that political quarrels should be faithfully reflected in the pattern of sport, who is to say that the next nation to be ostracized will not be the Soviet Union or the United

States, not to mention the United Kingdom (which is about to supply a handful of helicopters to South Africa)?

## 100 Years Ago



### NOTES

IN the second report of the Royal Sanitary Commission, just published, the Commissioners appear to be under the impression that no branch of science other than that of medicine is to any great extent involved in sanitary questions, and therefore recommend that the 4,000 medical men appointed under the Poor-law Board should be the inspectors under the proposed new sanitary department; mentioning, however, the probability of a variety of officers being requisite for scientific purposes only. We think that, in order to carry out sanitary reform efficiently, the new department should have the means of consulting the highest authorities in most of the branches of physical science, and here, as in many other cases, we see the necessity for a Board of Science, whose duty it should be to advise the Government on all scientific questions.

WE regret to state that the work at the new buildings at Burlington House for the learned societies has come to a standstill, owing, we are informed, to the failure of the contractors.

From *Nature*, 3, 393, March 16, 1871.

## OLD WORLD

# Government Survey Raises Unemployment Spectre

THE spectre of unemployment that has been haunting qualified scientists and engineers in the United States during the past few years is beginning to raise its head in Britain. It is unlikely to be exorcised by the findings of a survey carried out by the Government Statistical Service and published this week by the Department of Trade and Industry (*Persons with Qualifications in Engineering, Technology and Science, 1959-1968*, HMSO, £2.25). Although the survey suggests that only about 2 per cent of the scientists and 0.5 per cent of the qualified engineers were out of work in 1968, Mrs Joan Cox, who directed the study, said at a press conference this week that Britain may be "producing more graduates than there are traditional jobs available".

The basis for this opinion is not so much that scientists and engineers form a disproportionate percentage of the unemployed—in 1968 2.4 per cent of the total British workforce was unemployed, for example—but that new graduates are experiencing increasing difficulty in finding suitable employment. Consequently, many are ending up in jobs for which they are patently unsuited. University appointments officers have, indeed, been pointing out with increasing alarm that they are having difficulty in finding jobs for many of the science and engineering students on their books and tales of scientists returning to Britain from abroad to find little prospect of acceptable employment are becoming depressingly commonplace. Whether the root cause lies in overproduction of qualified manpower or in the present economic climate which makes industry reluctant to spend more money on costly research and development is a moot point, and the team of economists who conducted the survey seeks to provide no answer.

Described by Mrs Cox as a "coherent and dynamic synthesis of data" which will form the basis for policy decisions, the survey is packed with information. It shows, for example, that the total stock of qualified scientists and engineers (QSEs) in Britain increased by 60 per cent between 1959 and 1968, from 255,000 to 403,000. But, unlike trends in the United States, the pool of scientists expanded faster than the pool of engineers (65 and 52 per cent respectively), so that in 1968 Britain boasted equal numbers of qualified scientists and engineers. Of the grand total, 347,000 were economically active, 185,200 of these being engineers and 162,600 scientists. The so-called eco-

nomically inactive stock consists chiefly of retired people and married women living at home (Women's Lib may disagree with the analysis of economic inactivity)—and the greater percentage of active engineers reflects the paucity of women attracted to careers in the applied sciences.

A much publicized and, it seems, much misunderstood factor bearing on the stock of qualified manpower in Britain is the so-called brain drain. The survey shows, however, that as far as scientists are concerned, the brain drain has been something of a myth, for on balance there has been a net migration to Britain of qualified scientists during the past decade (see Table 1). The net loss of scientists from Britain in 1967 and 1968 is explained in part by the rapid expansion of the Apollo project and this factor again accounts for some of the drift of engineers away from Britain in the late 1960s. But the overall emigration of engineers during the past decade is a much more worrying problem. Table 1 shows that the immigration/emigration balance is tipped heavily in favour of emigration—the net emigration of engineers from Britain amounted to some 15,000 between 1958 and 1969. Coupled with the fact that the proportion of engineers in the domestic stock of scientists and engineers is already much smaller than that in many other industrialized countries, this exodus throws up some important considerations.

Unfortunately, the survey makes no mention of the academic qualifications of the emigrating scientists and engineers and it gives only scant information about their fields of activity. Few conclusions can therefore be drawn about the reasons for the exodus. The Jones and Swann reports, of course, both pointed to a few possible reasons, and both put considerable emphasis on the

fact that monetary and other incentives for engineers in the United States are much greater than those offered in Britain. This survey conflicts, however, with the Jones report about the size of the brain drain. The Jones report tended to overestimate the extent of the problem because it did not have at its disposal reliable figures for foreign-born immigrants—these have only become available since the 1966 census.

An important reason for the flow of engineers out of Britain is the lack of job opportunities on the domestic market. This factor, for example, was instrumental in the closure of an office run by Management Selection Ltd in New York, designed to attract expatriate British scientists back to jobs in Britain. MSL decided in 1969 to close down the office and to concentrate instead on attempting to make firms in Britain more aware of the possibilities of employing returning scientists.

The lack of job opportunities is also reflected in the level of unemployment among QSEs in Britain. Although the present situation and the future prospects are much worse in the United States (see *Nature*, 229, 448; 1971), unemployment in Britain is becoming increasingly a cause for concern. Between the 1961 census and the 1966 census—the only years for which reliable figures are available—the proportion of unemployed engineers increased from 0.62 to 1.08 per cent of the total stock, while out-of-work scientists formed 0.77 and 1.27 per cent of the stock in these two years respectively. Table 2 shows that these rates of unemployment were steadily drawing closer to the overall unemployment rate in Britain and, on this basis, the team which conducted the survey estimates that unemployment among QSEs as a whole had reached 2.4 per cent by 1968.

But this figure conflicts with the results of a survey carried out in 1968,

**Table 1** Balance of Migration of QSEs

|      | Engineers and technologists |            |         | Scientists |            |         |
|------|-----------------------------|------------|---------|------------|------------|---------|
|      | Emigrants                   | Immigrants | Balance | Emigrants  | Immigrants | Balance |
| 1958 | 2,725                       | 1,785      | -940    | 2,885      | 3,405      | +520    |
| 1959 | 2,525                       | 2,025      | -500    | 2,855      | 3,935      | +1,080  |
| 1960 | 2,530                       | 2,110      | -420    | 2,695      | 4,100      | +1,405  |
| 1961 | 2,430                       | 3,215      | +785    | 3,315      | 4,035      | +900    |
| 1962 | 2,735                       | 3,025      | +290    | 3,100      | 4,285      | +1,185  |
| 1963 | 3,065                       | 2,240      | -825    | 3,490      | 4,220      | +730    |
| 1964 | 3,750                       | 2,355      | -1,395  | 3,880      | 3,910      | +30     |
| 1965 | 4,050                       | 3,125      | -925    | 4,240      | 4,880      | +640    |
| 1966 | 5,255                       | 2,760      | -2,495  | 4,470      | 4,720      | +250    |
| 1967 | 6,180                       | 2,440      | -3,740  | 4,895      | 4,160      | -735    |
| 1968 | 4,945                       | 2,865      | -2,080  | 4,995      | 4,970      | -25     |
| 1969 | 4,685                       | 2,930      | -1,755  | 4,830      | 5,039      | +190    |



**Table 2** Assessment of "Expected" Unemployment among QSEs 1959 to 1968

|                   | Great Britain percentage rate <sup>1</sup> | QSEs <sup>2</sup> out of employment | Engineering and technology |                     | QSEs <sup>2</sup> out of employment | Science QSE percentage rate | Ratio QSE : GB rate |
|-------------------|--------------------------------------------|-------------------------------------|----------------------------|---------------------|-------------------------------------|-----------------------------|---------------------|
|                   |                                            |                                     | QSE percentage rate        | Ratio QSE : GB rate |                                     |                             |                     |
| 1959              | 2.2                                        | 1,215 (38)                          | 0.97                       | 0.443               | 1,215 (88)                          | 1.21                        | 0.550               |
| 1960              | 1.7                                        | 980 (28)                            | 0.75                       | 0.443               | 995 (85)                            | 0.94                        | 0.550               |
| 1961 census       | 1.4                                        | 845 (20)                            | 0.62                       | 0.443               | 860 (105)                           | 0.77                        | 0.550               |
| 1962              | 1.8                                        | 1,355 (30)                          | 0.94                       | 0.521               | 1,350 (123)                         | 1.15                        | 0.640               |
| 1963              | 2.5                                        | 2,260 (90)                          | 1.50                       | 0.598               | 2,275 (197)                         | 1.83                        | 0.730               |
| 1964              | 1.7                                        | 1,805 (56)                          | 1.15                       | 0.676               | 1,820 (228)                         | 1.39                        | 0.820               |
| 1965              | 1.4                                        | 1,715 (52)                          | 1.05                       | 0.753               | 1,750 (160)                         | 1.27                        | 0.910               |
| 1966 census       | 1.3                                        | 1,845 (44)                          | 1.08                       | 0.831               | 1,900 (145)                         | 1.30                        | 1.000               |
| 1967              | 2.3                                        | 3,735                               | 2.09                       | 0.909               | 3,550                               | 2.30                        | 1.000               |
| 1968              | 2.4                                        | 4,390                               | 2.37                       | 0.986               | 3,905                               | 2.40                        | 1.000               |
| 1967 <sup>3</sup> |                                            | 1,100 (80)                          | 0.62                       |                     | 3,040 (202)                         | 1.97                        |                     |
| 1968 <sup>3</sup> |                                            | 940 (115)                           | 0.51                       |                     | 3,200 (339)                         | 1.97                        |                     |

<sup>1</sup> Wholly unemployed less temporarily stopped and school leavers.

<sup>2</sup> New graduates seeking permanent employment are shown in brackets.

<sup>3</sup> Unemployment figures adjusted to allow for emigration which is over and above the normal international circulation.

which produced the finding that unemployment among QSEs was only 0.4 per cent in that year. The team therefore decided that emigration ought to be taken into account. A "normal international circulation" of QSEs, measured by the lowest point on the fluctuating curve of immigration, was defined as the flow of scientists and engineers for business or family reasons, and this circulation, the team argues, would be unaffected by economic considerations. Emigration above this circulation level can be considered excess emigration, and its chief effect on domestic unemployment lies in the fact that it consists chiefly of those QSEs who otherwise might have found themselves out of a job. The much more respectable unemployment figures shown in the bottom two lines in Table 2 are derived by subtracting this excess unemployment from the expected unemployment. Luckily, the excess emigration level was smaller in 1967 and 1968 than the expected unemployment, and the committee was saved the embarrassment of having to explain a negative unemployment rate.

Another indication of the lack of job opportunities for QSEs in Britain is the finding that the number of people with degrees who are employed as technicians almost doubled between 1965 and 1968, rising from 3.8 per cent to 6.0 per cent of all technicians. There was also a marked, but welcome drift away from employment in strictly research and development functions during the 1960s, but whether this drift reflects a desire to leave the laboratory bench for the office desk or simply the lack of opportunities in research and development is open to debate. In any case, the movement underlines the arguments put forward in the Dainton and Swann reports for a broader education for scientists and engineers, so that their

mobility is enhanced, and consequently the move away from research does not require the learning of a completely fresh set of skills.

The survey also shows that in 1968, manufacturing industry employed about 35 per cent of the economically active stock of QSEs, and industry as a whole increased its share of scientists and engineers by 0.8 per cent between 1965 and 1968. Engineers and technologists also parted company with many of their scientific colleagues on graduation, because while 43.7 per cent of engineers were employed by manufacturing industry in 1968, only 25.4 per cent of the scientists had found their way to industry. Education was, of course, the chief employer of scientists, taking 36.3 per cent of the economically active group.

#### COMPUTER POLICY

### Prognosis for ICL

MORE gloom about the future of International Computers Limited was voiced last week. Mr Kenneth Warren, a member of subcommittee A of the Select Committee on Science and Technology, said that some members of the subcommittee were wondering whether ICL will still exist in two or three years' time. Appearing before the subcommittee was Mr David Howell, Parliamentary Secretary to the Civil Service Department, and he was questioned among other things on the wisdom of the Government's reliance on ICL for its supply of computers. Mr Warren remarked that there is no alternative source in Britain. He thought that the Civil Service Department and the Department of Trade and Industry should therefore take a mutual interest in the health of ICL. Mr Howell replied that the interest is there, but the subcommittee did not seem convinced.

Members of the subcommittee were clearly still unhappy about the Conservative Government's policy towards the computer hardware industry, announced by Sir John Eden at the previous session (see *Nature*, 230, 4; 1971). Withdrawal of Government support from ICL bothered the subcommittee, although Mr Howell insisted that "withdrawal" is too strong a word. It is true that no more payments will be made to ICL under the merger agreement once the legal obligations have been fulfilled, but the policy of buying computers from ICL through the single tender system will continue (the new policy not to apply this system to the purchase of really large computers will make little difference, Mr Howell said), and the Government has promised to look sympathetically at the notion of awarding development grants to ICL.

But the subcommittee was not persuaded to buy a pig in a poke. It wanted to know precisely what was meant by development contracts in this case. Was it just subvention of ICL's expenditure on research and development by another name? Mr Howell was not able to help. Mr Warren said that the question of development contracts was "an urgent, desperately important, question"; he hoped that "state of the art" development contracts would become a feature of the Government's relationship with ICL. Echoing these remarks, Mr Airey Neave, chairman of the subcommittee, said that a definition of Government policy on development contracts is very important and urgently required. Next week the subcommittee will be asking representatives of ICL how bright the future looks from their side of the fence.

Later in the session, Mr Howell told Mr Ian Lloyd of the subcommittee that the data processing requirements of the House of Commons had not yet been

considered. Mr Warren added that there is an urgent need for such a system. "We need to get with it a bit more in this place," he said. "It would help us to behave rationally, not like sheep, in the voting procedure."

#### SPACE POLICY

### Goods Without Prices

A NATIONAL satellite for direct television broadcasting, participation in the post-Apollo programme and vigorous pursuit of international participation in applications satellites are among the many tempting fruits offered last week to the Select Committee on Science and Technology by the British Aircraft Corporation. Unfortunately, BAC neglected to put a price tag on many of the goods, and the select committee was left in some doubt about the size of Britain's potential shopping bill if all the choices offered by the vendor of space technology were taken up. In many respects, the BAC memorandum to the committee was a public relations exercise, extolling the virtues of space research and pointing the way towards possible goals without regard to cost. Indeed, most of the proposals are unlikely to be greeted with much warmth in Whitehall because they fly directly in the face of present British thinking on launcher development.

The thrust of BAC's argument is that unless British space policies take full account of likely developments in space research, British companies are likely to miss out on the opportunity of securing potentially valuable international contracts. Notably lacking among the proposals was discussion of the merits of strictly scientific satellites—these "may be done either as national or international projects in the light of the particular scientific requirements and the funding available"—and the argument was geared chiefly to applications satellites and launcher development.

Fundamental to BAC's thinking is British participation in the post-Apollo programme. In spite of the fact that the total cost of the project has not yet been worked out, and that the British government has decided not to take part in further study of European participation in the post-Apollo project, BAC had prepared for the select committee a vigorous argument for British participation in the project. Four principal reasons for participation were invoked by the company—that it would secure guarantees from NASA for the launching of UK satellites, that the cost of these launchers would be known and that launch costs and the cost of applications satellites would be reduced.

Without some cost estimates to back up these arguments, however, relative savings cannot be calculated, or even

guessed at, and until the whole project is more clearly defined, the British government is unlikely to change its attitude. But BAC has, on its own initiative, signed an agreement with the North America Rockwell corporation to participate in feasibility studies of the shuttle programme, and the British government has agreed to provide some of the costs of the study.

The post-Apollo programme is important to BAC's thinking on British space policy because it would provide the necessary guarantees for launchers for domestic and European satellites (apart from providing valuable development contracts for British space companies). Such guarantees would obviate the difficulties that may arise over American launching of European communications satellites, which may conflict with NASA's commitments to Intelsat, and would open the way for BAC's second chief proposal—a British national communications satellite for direct television broadcast.

The idea is that a national satellite, in geostationary orbit over the equator, could be used to beam broadcasts over the whole of the UK. The small size of the area covered would, the memorandum points out, enable high power density to be achieved on the ground with a relatively small satellite. Unfortunately, however, the arguments used by BAC to justify development of such a satellite are a little thin, and apart from the assurance given by Mr Donald Rowley, director of BAC Space Systems division, that it would cost about a quarter of the proposed expenditure on completing coverage of BBC2 in the UK, costs were given only passing reference.

Briefly, BAC considers that a British stake in communications satellite technology is important to prevent domination by the United States and the USSR. "Some of the activities such as educational and entertainment television may well be used directly to influence the countries receiving them," the memorandum warns, and some counterbalancing of American and Russian influence by "European culture" is desirable. Moreover, the proposal for a British national satellite would ensure "the provision of secure communications links for government purposes which would be safe from industrial action and from insurrectionists". With luck, the government is not sufficiently concerned about insurrection to be moved by such arguments, and a more convincing case could have been drawn up for such projects on the basis that they would have good export potential, and their development would give a welcome boost to domestic space companies. But that may, perhaps, have been rather too much like obviously touting for business.

#### PASTEUR INSTITUTE

### Monod Gets Top Job

JACQUES MONOD, winner of the Nobel prize for medicine in 1965, has been elected Director of the Pasteur Institute. He replaces the microbiologist Pierre Mercier, who has held the position since 1966. Monod, 61, has been chief of the department of cellular biochemistry at the Institute since 1954, but his elevation to the directorship is nevertheless something of a surprise.



There are many facets to the character of Jacques Monod; scientist, philosopher, left-wing intellectual. Although none would deny his brilliance in the first role—his fundamental work on allosteric proteins earned him a third share in the 1965 Nobel prize for medicine in the company of André Lwoff and François Jacob—his other activities have not met with such widespread approval. In particular, many eyebrows were raised in disapproval of his vocal participation in the May riots of 1968, where he urged the students to fight for realistic demands. Monod's fellow scientists should have no such reservations about his new responsibilities, but many must hope that the demands on his time will not now be so great as to preclude the hope of further experimental and theoretical breakthroughs such as the operon concept of 1961, an idea which illuminated the whole field of molecular genetics.

The Pasteur Institute manufactures a wide range of pharmaceuticals; the organization is non-profit-making, however, for the profits from sales are ploughed back into the basic research for which the Institute is justly acclaimed.



## ARCHAEOLOGY

## Missing Fort Found

from a Correspondent

THE discovery last summer of remains of a Saxon fort in the centre of Dover provides the missing link in the chain of nine coastal forts listed in the Roman *Notitia Dignitatum*. Eight of the nine forts contained in the civil and military list, which was probably compiled in the fourth century, were discovered long ago at sites from Norfolk to the Isle of Wight, but the site of the last one, named Dubris, remained elusive. The first full account of the finding of this fort has now been published in the spring issue of the *Kent Archaeological Review*, by B. J. Philp, the chief excavator.

Although the exact location of Dubris was not known until its south wall was unearthed last July, Sir Mortimer Wheeler predicted as long ago as 1929 that the fort probably lay underneath the present market square. Several attempts to establish its existence by excavating in the area proved abortive, however, and a number of archaeologists were even forced to suggest that the fort may never have been situated in Dover. The building of a new road through the centre of the town, however, provided a good opportunity for a rescue-research dig where buildings had been demolished. The fruit of this labour was the discovery of two separate forts, one dating from the second and the other from the third century AD. Twenty thousand archaeological finds were also unearthed during the excavation.

The second century Classis Britannica fort may have been deliberately pulled down to make way for the larger and more strongly fortified Saxon shore fort, and the aim of further excavation this year will be to look at such a possibility. The Saxon fort seems to be trapezoidal in shape, with the walls meeting at an angle of about 100 degrees, and this shape may account for the failure of previous attempts to find any part of the structure. Other late Saxon shore forts, at Burgh Castle (Norfolk) and Bradwell (Essex), were also trapezoidal in shape, while earlier forts, such as Reculver, were rectangular. Otherwise, the Dubris fort matches Reculver in size—the west wall, for example, is at least 45 m long.

As far as the Classis Britannica fort is concerned, this discovery, Mr Philp suggests, proves the existence of a major naval base at Dover during at least the second century AD. Associated with the fort, the excavations unearthed fragments of tiles bearing the initials CLBR, representing the Classis Britannica, of British fleet, and a delicate bronze hand holding an orb surmounted by a dove-

like eagle. This is apparently a victory symbol that was mounted on a wooden frame for ceremonial occasions.

## ENVIRONMENT

## Advanced Planning

from a Correspondent

PLANNING is now well advanced for the United Nations Conference on the Human Environment, to be held in Stockholm in June 1972. This was apparent at the meeting of the 27 nation Preparatory Committee held in Geneva from February 8 to 19. By the end of the general debate, one thing was already apparent—whereas a year ago at the first meeting of the committee, it seemed as though the United Nations might be pioneering in this field, the conference now appears as the logical product of genuine governmental interest. This meeting was lively, constructive and above all businesslike. One factor making for a useful meeting was the clarity and forcefulness of Mr Maurice Strong, seconded from his position as head of the Canadian International Development Agency. The Stockholm Conference, Mr Strong explained, is conceived on three levels: conceptual; "action plan" and "action completed". Documentation will be in line with this approach. The chief document at the first level will thus be a "Report on the State of the Environment" summarizing individual reports submitted by governments, United Nations agencies and other organizations. At the two other levels will be—apart from these official reports—case studies of especially valuable experience and "basic papers" covering specific aspects of subjects not adequately dealt with elsewhere.

Delegates at Geneva this time were impressed with the way in which these preparations have been pushed ahead. They were, however, obviously concerned that the list of topics suggested for discussion was too long, and during the two week meeting a good deal of streamlining took place. The six main subjects areas suggested were, however, retained: the planning and management of human settlements for environmental quality; the environmental aspects of natural resource management; pollution identification and control (in the broadest sense); the educational, social and cultural aspects of environmental issues; the relationship between development and environment; and the implications of proposed actions in respect of (new) international organizations or institutions. With regard to the last of these items, the government delegates were unanimous in insisting that there was no need to set up a new United Nations organization to deal with environmental problems. As Mr Hoveida, Iran's

deputy foreign minister, put it, the United Nations should do its own family planning in this respect.

It has also been decided to set up working groups to study five subjects on each of which it is hoped that action might be possible as a result of the Stockholm conference. These topics include marine pollution, erosion and soils conservation (with especial reference to wetlands and oceanic islands), monitoring and the proposed Declaration on the Environment, regarded as one of the chief objectives of the conference. The organizers have by now abandoned earlier proposals to treat in the same way the compilation of a "register of chemical compounds" obviously a source of considerable embarrassment to several governments.

On only one subject did the delegates appear to feel somewhat uneasy, and that was the participation of the less developed members of the world community at Stockholm. A glance at the empty chairs around the committee's table showed that hardly any of the "third world" countries that could have been represented were in fact present. For example, Africa was represented only by Zambia and Latin America by Brazil and Mexico, although both the Iranian and Indian delegates made full contributions to the debates. Evidently it will take a good deal to persuade the governments of many of these countries that concern for the environment is not just a luxury nor even a disease of the rich countries.

## PHYSICS

## Surfaces Selected

SURFACE physics is to be treated by the Science Research Council as a selected area, deserving special support for at least five years. This is the chief recommendation of a panel set up by the SRC Physics Committee and it has now been accepted in full by the Council's Science Board (*The Physics of Surfaces*, Science Research Council, 1971).

The surface physics panel is one of about fourteen such bodies which have been set up by SRC committees to review activities in different areas of scientific research. The aim is to highlight new growth points which deserve special support—a report to the physics committee on plasma physics has, for example, already been published and other physics panels are looking into subjects such as ion implantation and amorphous materials (especially semiconductors). In line with the SRC's doctrine of selectivity, most of the grants awarded in selected fields will go to outstanding individuals and university departments.

The physics committee received re-



sponses from eighteen individuals and groups to its invitation to submit proposals for five year programmes of research in surface physics. The surface physics panel narrowed this number down to nine which it considers "timely and promising" and the physics committee has awarded grants totalling more than £200,000. Although the committee says that the door is not shut to other applications for support in surface physics, it is clear that the present level of support is considered to be sufficient.

The panel came to the conclusion that there is a particularly promising future for surface physics because of the many experimental and theoretical techniques which have been perfected during the past few years. Not least among these is the ability to produce and maintain ultra high vacua ( $<10^{-10}$  mm Hg) in large metal systems so that surfaces can be

created and examined under extremely clean conditions. Two of the chief surface probing techniques now in extensive use are low energy electron diffraction and reflexion high energy electron diffraction which are used respectively for examining bulk surfaces and films.

The panel sees the detailed understanding both of clean surfaces and of the changes produced by the sorption of gas and the deposition of other layers as a vital basis for future interdisciplinary research in fields such as gas-surface interaction chemistry and corrosion studies. The last of these is particularly topical because of the recently published Hoar report (*Report of the Committee on Corrosion and Protection*, HMSO, 1971; 80p) which highlighted the costs of corrosion and the enormous savings which could quite easily be made (see *Nature*, 230, 6; 1971).

## Parliament in Britain

### Higher Education

MRS MARGARET THATCHER, Secretary of State for Education and Science, said in reply to a question from Mr David Lane, that the government has no intention at present of issuing a green paper on higher education. She had earlier told Mr Edward Short that she cannot yet say when the government will be in a position to issue a statement on the restructuring of higher education, but that the government is now considering the problem of co-ordinating planning in relation to the polytechnics.

Mr Judd suggested that a major social problem in Britain is that many of those who go through the higher education system find difficulty in communicating with the majority of the population. He wondered whether exposure to work before going on to higher education would ensure that students make better use of their education and that they might later become more useful leaders of society. Mrs Thatcher agreed that there should be as much opportunity as possible for spending some time in employment between school and university, but pointed out that it would not be possible to legislate for this. (Written and oral answers, March 4.)

### Advanced Gas Cooled Reactors

MR NICHOLAS RIDLEY, Under-Secretary of State, Department of Trade and Industry, said that so far no advanced gas cooled reactors have been sold abroad. Licences for the system have, however, been taken out by German and Japanese firms. Mr G. Elfed Davies had asked what have been the benefits of development of the advanced gas cooled reactor in terms of overseas business. Mr Ridley assured him that development of AGR technology has been instrumental in securing overseas business in oxide fuel manufacture, reprocessing and ancillary equipment. (Written answers, March 1.)

### Students' Grants

STUDENT grants are at present under review to determine the rates payable from September 1, 1971. But Mr David Clark and several other members asked whether there could be an interim increase to obviate the effects of inflation since the last increase. Mrs Thatcher replied that the review procedure for negotiating student grants is strictly laid down, and should not be broken. Mrs Thatcher also said that the review applies to first degree or equivalent courses at universities, colleges of education and technical colleges. (Written and oral answers, March 4 and 5.)

## Miscellaneous Intelligence

It now seems that the age of a pilchard can be determined, from a measurement of the size of the bony structure known as an otolith found in its ear, as accurately as the age of a tree can be told from its growth rings. The Division of Sea Fisheries at Cape Town, which has a straightforward commercial interest in the pilchard fishery off the coast of South Africa, has been able to collect enough otoliths at different seasons of the year to show that this structure is increased in size by one layer each year. What seems to happen is that the otolith grows steadily, but that the material added during the winter months is transparent and that added during the summer is opaque. This all fits in well with the way in which the central region of each otolith suggests that it was formed during the winter, which is when the pilchards would have been larvae. As luck will have it, the correlation between the length of a fish and the length of its otolith is such as to warm the heart of a statistician, from which it follows that the length of a pilchard can be taken as a fairly unambiguous function of its age  $t$  in years given by  $30.6(1 - e^{-0.2247(t+1.505)})$ . On this basis, the length of the largest possible pilchard off the coast of South Africa should not exceed 31 cm in length. This is something for local fishermen to work towards.

THE bookshop at the Imperial College of Science and Technology is less mysterious than similar organizations elsewhere. It seems also to be thriving. Turnover increased between 1969 and 1970 from £39.6 thousand to £57.7 thousand. The price paid for the books sold for 1970 seems to have been 77 per cent of turnover, which suggests that

the bookshop has some way to go in extracting better discounts from its suppliers. The operation as a whole seems to have finished up with a profit of more than 10 per cent, something that even successful bookshops would be proud of these days. The various student unions linked with Imperial College benefited to the tune of £4,000 in 1970. In this as in many other respects, Imperial College seems to be doing well—indeed, the college as a whole managed to make a profit on most of its halls of residence and to increase its assets from £29.5 million to £32.3 million. At this rate, the college will quickly become what some consider it to be already—the tail that wags the dog.

THE Department of Education and Science, in the January issue of *Trends in Education*, has some gentle propaganda for the benefit of aircraft as a means of studying the environment. Mr J. C. Jennings, an inspector of schools who began life as a historian, argues the case for hiring aircraft at £3.00 per pupil hour to carry out a variety of observations from the identification of old patterns of agriculture and motte and bailey castles to the study of meteorology. On the principle that there is nothing new under the Sun, many past students of the University of Edinburgh will point out that the use of aircraft for student instruction was pioneered by the late C. T. R. Wilson, who was a great enthusiast for transporting students about Scotland in military aircraft. All those who travelled with him were aware that no amount of air sickness on their part would diminish his enjoyment of a journey.

## NEW WORLD

## Laissez-faire on the Breadline

by our Washington Correspondent

THE 50,000 scientists and engineers thrown out of work by cutbacks in the aerospace industries need not expect the present Administration to create new jobs appropriate to their skills but must wait for an upswing in the economy to lift them out of the dole queues. Although plans for retraining aerospace scientists and converting their expertise to domestic problems are devised almost daily in Congress or government departments, the Administration is responding only with token gestures and expressions of concern, apparently in the belief that the expected economic growth will be a sufficient cure for unemployment. Nor is there any eagerness to be seen to give privileged treatment to scientists and engineers, among whom unemployment is at present slightly less than the national average rate of 5.8 per cent.

The Administration's impassive attitude towards jobless scientists has been made clear on several occasions in the last few months, notably at the hearings held last November before the House subcommittee on Conservation and Natural Resources to consider how the skills of the aerospace industry could be applied to renovating the environment. Murray L. Weidenbaum, Assistant Secretary of the Treasury for economic policy and a former corporate economist for Boeing, told the committee that it would be against the national interest to see how the aerospace industry could be adapted to solving pollution problems; efficiency and the public would be best served, he said, by letting all kinds of companies, including the aerospace companies, compete for the "environment market". Edward E. David, the President's science adviser, confirmed Weidenbaum's posture by indicating to the committee that the Office of Science and Technology had no definite plans for helping the aerospace industry diversify into the environmental area.

The Administration's scepticism towards retraining plans for scientists was reaffirmed at a private conference held last week in Washington for the heads of professional societies and representatives of universities and industry. Convened by the Department of Labor and the Office of Science and Technology, the conference followed by a few weeks the introduction of two bills by Democrats in Congress that would appropriate sums of up to \$500 million for the retraining of job-

less scientists and engineers. But no grand new initiatives on the part of the Administration were announced at the conference, the chief purpose of which was apparently to exchange information and express sympathy.

"The President is concerned about unemployment in general, and especially among scientists and engineers," David said at a briefing held after the conference, but had little further comfort to offer. Malcolm R. Lovell, Assistant Secretary of Labor for manpower made clear his opinion that "the Federal Government is doing the major things it is appropriate for it to do today" and that as the economy resumes its growth the demand for science and engineering skills will increase. Lovell was confident that the period of layoffs has just about ended so the unemployment situation will not get any worse. Both he and David pointed to such indirect guarantees of an improved job market as the 8 per cent increase in Federal funds for research and development budgeted for the coming financial year, as well as long range projections by the Bureau of Labor Statistics that there will be substantial shortages of certain kinds of scientists, notably physicists and chemists but to a lesser extent of engineers, by the 1980s (see table).

Apart from a national job registry

at Sacramento which now has 6,000 names on its books, the only direct aid for jobless scientists Lovell and David had to discuss was a plan for paying county and state governments to retrain between 400 and 600 unemployed scientists and engineers in public service jobs. The plan is an experimental project, to be administered by the Departments of Labor and of Housing and Urban Development at a cost of \$1.2 million. This mouse is the fruit of mountainous labour within the Department of Housing and Urban Development, a project by which two months ago it was proposed to retrain 3,000 jobless aerospace scientists, as well as a similar number of returning Vietnam veterans, in skills relevant to urban problems. The Executive has now pared these plans down to a size it considers appropriate.

Another government agency whose conversion schemes have been ignored at higher levels is the National Science Foundation, which asked in its original 1972 budget plans for funds to retrain jobless aerospace scientists in universities. This request was turned down by the Office of Management and Budget. At present the foundation's only effort in the field is a \$136,000 grant that provides for retraining 15 people in computer engineering. But the NSF has some 30 unsolicited proposals prepared,

**Table 1** Occupational Employment, 1968, and Projected Requirements, 1980, for College Graduates

| Occupation                         | Estimated 1968 employment | Projected 1980 requirements | Per cent change | Supply estimated to be           |
|------------------------------------|---------------------------|-----------------------------|-----------------|----------------------------------|
| Chemists                           | 130,000                   | 200,000                     | 55.7            | Significantly below requirements |
| Counsellors                        | 71,000                    | 107,000                     | 49.8            |                                  |
| Dietitians                         | 30,000                    | 42,100                      | 40.3            |                                  |
| Dentists                           | 100,000                   | 130,000                     | 31.7            |                                  |
| Physicians                         | 295,000                   | 450,000                     | 53.1            |                                  |
| Physicists                         | 45,000                    | 75,000                      | 63.9            | Slightly short of requirements   |
| Engineers                          | 1,100,000                 | 1,500,000                   | 40.2            |                                  |
| Geologists and geophysicists       | 30,000                    | 36,000                      | 20.6            |                                  |
| Optometrists                       | 17,000                    | 21,000                      | 23.5            |                                  |
| Architects                         | 34,000                    | 50,000                      | 47.1            | In balance with requirements     |
| Lawyers                            | 270,000                   | 335,000                     | 22.7            |                                  |
| Pharmacists                        | 121,000                   | 130,000                     | 7.0             | Significantly above requirements |
| Mathematicians                     | 70,000                    | 110,000                     | 60.5            |                                  |
| Life scientists                    | 168,000                   | 238,000                     | 40.8            |                                  |
| Teachers, elementary and secondary | 2,170,000                 | 2,340,000                   | 7.8             |                                  |

Source: College Educated Workers 1968-80. Bureau of Labor Statistics, 1970.



or in the pipeline, for schemes to retrain jobless scientists. "It is conceivable we will be able to support a few of them," an NSF official said last week, but this can only be at the expense of other projects since the NSF has no special funds for conversion projects.

No congressman is harmed among his constituents by expressing concern in unemployment and several bills designed to reduce unemployment in various ways have been introduced. Two which concern scientists and engineers specifically are those proposed by Senator Edward M. Kennedy on January 26 and by Congressman Robert N. Giaimo and John W. Davis, on February 10. Both bills are based on a version introduced by Kennedy last year which called for an expenditure of \$450 million in converting the orientation of the economy from defence to civilian needs. Kennedy has now prepared an expanded version of his bill, cosponsored by fifteen other senators, which asks for \$500 million over three years to achieve conversion of the nation's scientific and technical manpower. The bulk of the programmes called for in the bill would be administered by the National Science Foundation, with the Small Business Administration overseeing a number of programmes designed to help small research and development firms convert to civilian operations.

The bill asks Congress to establish three national policies designed to create full employment among scientists and engineers; first, that scientists must have continuing opportunities for employment "in positions commensurate with their professional and technical skill"; second, that Federal support of civilian research and development should be raised to and maintained at the same level or above that of defence related research and development; and third, that the total Federal investment in science and technology should continue to grow at the same rate as the gross national product.

Specific programmes authorized by the bill include \$225 million (over a three year period) to be distributed by the NSF in the form of conversion fellowships to jobless scientists and engineers enabling them to retrain in other jobs. Another \$65 million would enable the NSF to support "community conversion corporations", non-profit organizations located in communities particularly affected by defence cutbacks, the function of which would be to attract civilian research and development funds and provide on-the-job retraining for scientists and engineers. A third NSF programme would dispense \$45 million to state and local governments to carry out conversion programmes, and another \$45 million would be allotted to the Small Business Administration

for grants to assist small scientific firms in their conversion. Altogether some 20,000 people would be affected.

Reintroducing the bill this January, Kennedy spoke of the "immense human suffering and personal tragedy" as well as loss of national investment involved in scientific unemployment, adding that "individual scientists who interrupt their careers—as many are now forced to do in seeking other employment—may find it impossible to re-enter the scientific job market, in view of the rapidity with which new scientific knowledge is generated". Strong words were also used by Congressman John W. Davis, who introduced the House version of the bill; government policies, he said, "have allowed one of our most important national resources, our brain trust of scientists, engineers and technicians, to wither on the vine". The Davis-Giaimo bill, similar to the Kennedy bill except that it asks for expenditures of only \$450 million, calls for science and technology to be converted not simply to serve the consumer needs of society but also to the resolution of social ills; "science must serve society in coping with such problems as unemployment, poverty, crime, race relations, pollution, nutrition, housing, health care, transportation, education and social alienation".

#### HARVARD

### Investment Policies

THE Harvard Committee on University Relations with Corporate Enterprise under Professor Robert W. Austin of the Harvard Business School has spelled out a judicious line to follow on the university's policy as a stockholder. The committee was appointed in April 1970 after a bout of discussion of the proposal that Harvard (and other universities) should help to vote two independent stockholders to the board of General Motors. The report of the Austin committee is unlikely to provide an automatic means of resolving future conflicts of this kind but it should at least help to ensure that future arguments are well informed.

The starting point for the committee's recommendations is the view that universities must put first their commitment to free enquiry, that within this concept lies the freedom of individuals to take up the professions that seem to them to be right and that there are political as well as practical reasons why university funds cannot be used for purposes other than the pursuit of truth. That said, the committee affirms the right of universities to comment on political questions where these affect academic freedom or government financing, their duty to pay some heed to the condition of the community in

which they are sited and the need in relations with corporate enterprise to balance "the potential drying up of financial resources" against the danger that the academic climate could be so heated up that students and teachers were distracted.

The committee says that the first consideration when a university is seeking to invest money is to seek the best return. Not merely is this the only simple policy but there is a need to meet as fully as possible the growing cost of operating universities if only to retain their independence. The committee says, indeed, that in investment policy, the rule to seek the maximum return should be abandoned only in the light of the university's duty "to the more or less immediately surrounding community". But the committee does agree that there are some kinds of financial investments which should be excluded, although it emphasizes that the ideal of investment security is hard to attain. It would, however, avoid investments in tobacco companies, South African corporations and corporations that practise racial or other forms of discrimination. "If the university would not consider buying stock in gambling houses, even where legal and however attractive financially—as presumably it would not—it cannot close its eyes to the moral factors" in these examples.

Once a stockholder, a university is entirely within its rights to seek to influence a corporation, according to the committee. "It need not remain passive in the face of substantial evidence that the company is acting in an antisocial way." The committee does, however, consider that universities should stop short of litigation or the collecting of proxies to force particular policies on corporations and joining with other tax exempt organizations in policing the conduct of business corporations. "It is no answer that Harvard investments have not been big enough to give it an influential voice in corporate decisions. Certainly the university should vote its stock on occasions in favour of change for the symbolic effect of a great university's taking a position on a social problem."

But how can a university know its corporate view? The committee says that the way in which the university decided to vote for the General Motors management in the spring of 1970 may have been right, but was not seen by all members of the university to be responsible. What the committee suggests is that there should be a university officer with a small staff who would sift all suggestions about the non-financial aspects of Harvard's role as an investor, and make recommendations to the corporation of the university, ultimately responsible for the treasurer's activities.

## NEWS AND VIEWS

## Radio Search for Maffei 1 Draws a Blank

It is always exciting when something new is discovered on one's own doorstep, as it were. Last January a group in the United States reported that a recently discovered infrared object is almost certainly an elliptical galaxy at a distance of three million light years or so, which places it at the edge of the Local Group of galaxies containing the Milky Way (see *Nature*, **229**, 84; 1971). Known as Maffei 1 after its discoverer Paulo Maffei who reported it in 1968, the new object seems to be comparable in mass to the Milky Way. This makes Maffei 1 a significant new member of the Local Group that has hitherto gone unnoticed.

That the existence and nature of Maffei 1 have gone unrecognized for so long is not surprising, however. At a galactic latitude of  $-0.5^\circ$ , light from the object has to pass through the thickness of the Milky Way system before reaching the Earth, and the same goes for a second infrared object reported by Maffei in a similar direction. Obscuration by interstellar material trapped in the plane of the Milky Way is then a serious problem. Maffei 1 is therefore highly reddened, for the same reason that the setting Sun appears red through the thickness of the atmosphere. Hence Maffei 1 shows up well in the infrared, but is difficult to discern at visible wavelengths.

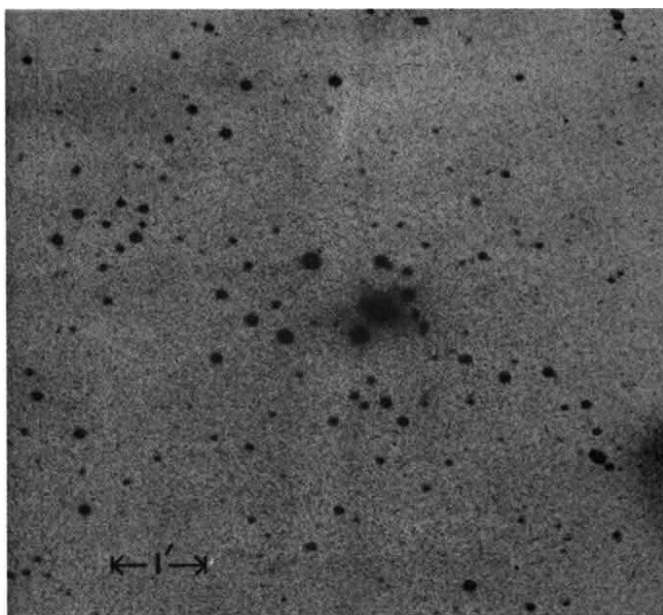
The story of Maffei 1 is taken a step further in this issue of *Nature* (page 103), from a radio astronomer's viewpoint. J. H. Oort, Leiden Observatory, gently points out that the absence of radio emission from Maffei 1 is going to be a problem. According to the original January article in *Astrophysical Journal Letters*, with an authorship reading like an extract from a *Who's Who* of astronomy, Maffei 1 is to be regarded, probably, as a giant elliptical galaxy at a distance between 0.3 Mpc (megaparsec) and 4 Mpc (1 parsec = 3.26 light years). A distance of 1 Mpc is accepted as a reasonable estimate (Spinrad *et al.*, **163**, L25; 1971). This places it at the edge of the Local Group. The mass of Maffei 1 is determined to be comparable to M 31 (the Andromeda nebula, at a distance of 700 kpc), and the Milky Way, the two normal spiral members of the Local Group. Elliptical galaxies normally do not contain any recognizable structure—their brightness decreases from the centre towards the edges, and the isophotes are circles or ellipses. Oort's most telling point then is that Maffei 1 is at least four magnitudes fainter at radio wavelengths than the faintest giant elliptical from which radio emission has so far been detected.

Oort's article has the distinction of being the first scientific article to come out of the new radio telescope array at Westerbork, Holland, officially opened on June 24 last year (see *Nature*, **226**, 1189; 1970). Chiefly the array of ten dishes is for detailed mapping of the radio sky, working on the aperture synthesis principle. What Oort is now presenting is a map of the 21 cm radio emission from an area of sky including Maffei 1. At the position of Maffei 1 there is no detectable radio emission greater than the order of 0.001 flux units. Taking the distance of 1 Mpc favoured by Spinrad *et al.*, this leads to an upper limit for the total radio energy of the order of  $10^{34}$  erg s $^{-1}$ .

This result may be an embarrassment because the giant

ellipticals that have been detected at radio wavelengths have significantly higher total radio energies. Oort has taken a look at two radio surveys which include an examination of elliptical galaxies, and points out that giant ellipticals from which radio emission has been detected have radio energies greater than  $5 \times 10^{38}$  erg s $^{-1}$ , four orders of magnitude greater than the upper limit for Maffei 1. But the surveys from which Oort has determined this figure were not sensitive to fluxes corresponding to total radio energies of less than of the order of  $10^{39}$  erg s $^{-1}$ . Clearly then, as Oort points out, the contrast between Maffei 1 and other giant elliptical galaxies has to be taken with a grain of salt; the population of giant ellipticals could well extend to members having radio energies of the order of  $10^{34}$  erg s $^{-1}$ , and this component would have remained undetected by the surveys. But Oort and his group at Westerbork have become sufficiently interested in the problem to continue their survey so as to achieve a sensitivity limit lower than the previous surveys.

Otherwise the evidence for Maffei 1 being a giant elliptical galaxy is compelling. Spinrad *et al.* base their conclusion on a battery of tests carried out at the Leuschner, Lick and Hale observatories. Briefly, the arguments are that the ellipticity of the system can be traced out in the isophotes to a radius of 3' or more; after allowing for reddening the spectrum is what one would expect from giant ellipticals; the infrared flux from Maffei 1 is comparable with that detected from the centre of M 31; and the spectrum contains absorption lines that are commonly found in the nuclei of certain types of galaxies including ellipticals. The distance estimate is thought to be too crude to be accepted as part of the evidence for the extragalactic nature of the object, but the rest of the evidence builds up a strong case. Infrared astronomy could well turn up further missing members of the Local Group.



The diffuse object is Maffei 1, observed with the Leuschner 30-inch telescope (plate L2, *Astrophys. J. Lett.*, **163**; 1971).



## SEA FLOOR SPREADING

**What Happens at Ridges?**

from our Geomagnetism Correspondent

ALTHOUGH it is widely accepted that as part of the process of sea floor spreading mantle material rises into the Earth's crust beneath mid-oceanic ridges, details of this phenomenon remain obscure. Almost nothing is known about conditions at the Mohorovicic discontinuity; and this ignorance makes it quite impossible to decide between the various conceptual models which have been proposed or even to say whether any of them is valid at all. Weertman (*J. Geophys. Res.*, **76**, 1171; 1971) has now come up with an ingenious explanation of what might happen at or below mid-oceanic ridges based upon the dislocation theory which is probably more familiar to solid state physicists than geophysicists. It is interesting that this is not the first time that dislocation theory has been applied to a geophysical problem. Weertman himself, for example, used it to explain the behaviour of water-filled crevasses in glaciers; and it was this which apparently led him to develop a similar theory for lava-filled cracks in the Earth's crust.

The general problem that Weertman has tackled is thus that of a liquid-filled crack in a horizontal elastic plate. Needless to say, the analysis is highly idealized; it could hardly be otherwise without a detailed knowledge of the Earth's interior. The density and elastic constants of Weertman's plate are thus constant and do not vary with depth; the liquid that fills the crack is incompressible; and the crack itself is both vertical and two-dimensional. In addition, the upper and lower surfaces of the plate are assumed to remain planar. Because of these simplifications Weertman's problem is rather different from the real Earth problem. But his aim is not to describe the actual phenomenon in detail but rather to set up a model which is feasible in spite of its limitations. There will be time later to assess the consequences of oversimplification when parameters relating to the Earth's crust and mantle are known more accurately.

The starting point for Weertman's analysis is a concept familiar in solid state physics—that an open crack may be regarded as a set of infinitesimal edge dislocations in the solid. The analysis itself involves a complicated mathematical treatment; but the results of it can be stated quite simply. It is first necessary to suppose that a pool of liquid mantle material accumulates at the base of the crust beneath a ridge. The most obvious mechanism for this would be that mantle convection currents bring some mantle rock into a position where temperature and pres-

sure are such as to liquefy it; but any mechanism would do as long as it produces the required liquid pool. It then turns out that as long as the crust is in a state of tension a bottom crack above the liquid pool will be "nucleated" into the crust—that is to say, there develops a crack into which liquid from the pools enters. Under the right conditions this bottom crack, filled with liquid, will grow in length until eventually it becomes sealed at the bottom and is thus cut off from the liquid pool. At this stage the crack is shaped like a tadpole with its tail pointing downwards. Because of its buoyancy the liquid-filled space will then rise until it reaches an equilibrium position near the surface of the crust, where the liquid solidifies.

In the meantime the remaining liquid pool will have produced another liquid-filled crack which again becomes sealed and which again rises. But this time the crack does not reach the surface of the crust but instead gets trapped by, and just below, the previous crack where it solidifies. The trapping process is then repeated at greater depths until the crust contains a solid

"dyke" of ex-mantle material. By this time, of course, the "dyke" has forced the crust apart—the ocean floor has spread a little. The whole cycle will then repeat itself over and over again, producing more and more crustal spreading. At first sight it may seem unlikely that all the new cracks will form immediately below the previous ones. In practice the cracks probably form within a short horizontal distance from each other; but because a crack increases the tensile stress in the region below it, the new cracks are more likely to form below the old ones.

This is not, of course, the first time that the solidification of lava into dykes has been suggested in this context. It has been implicit in much of the thinking about sea floor spreading; and Bodvarsson and Walker (*Geophys. J.*, **8**, 285; 1964) invoked it explicitly to explain the spreading of the mid-Atlantic Ridge where it crosses beneath Iceland. Weertman is at pains to point out, however, that the driving force for sea floor spreading is not the solidification of the lava as such but rather the tension which must exist in the crust before the process can take place.

**"Synchro-Compton" Emission from the Crab?**

THE discovery of the pulsar NP 0532 in the Crab Nebula provided a satisfying hint to astronomers of the origin of the energy driving this nebula outwards. With the almost universal acceptance of the oblique rotator model of pulsars, in which the pulsed radiation comes from a rapidly spinning neutron star surrounded by a strong magnetic field, it has now proved possible to explain in detail how the energy gets from the central pulsar to the wisps of matter in the nebula, and at the same time explain some of the observed polarization of the light from the Crab.

Next Monday, an article in *Nature Physical Science* by Dr Martin Rees of the Institute of Theoretical Astronomy shows that natural braking of the spin of the neutron star, occurring as a result of electromagnetic radiation at the pulsar frequency (30 Hz), ties in well with observations of the surrounding amorphous mass. The nature of this radiation should be the synchro-Compton emission which Rees presented recently as a possible source of the radiation seen in extragalactic radio sources. Although this can certainly provide the driving energy which keeps the nebula expanding, just as it can for larger, more distant sources (see, for example, *Nature* **229**, 368; 1971), the most immediate significance of this model is that it predicts exactly the polarization of the visible light from the source. Assuming a simple dipole field, an observer looking down on the

pole of the pulsar will see circularly polarized light, while one viewing the source in its equatorial plane will see linearly polarized light. Not only is the optical radiation from the Crab pulsar linearly polarized, but the presence of the "interpulse" has already been interpreted as an effect caused by observation of the pulsar from its equatorial plane. Ordinary synchrotron radiation would be completely inadequate to account for the degree of linear polarization observed in the Crab at both optical and radio frequencies. At present, it looks as if a combination of the oblique rotator model with the synchro-Compton emission process is the best explanation of the pulsar phenomenon, and, possibly, radio galaxies. But one note of caution should be sounded—the Crab Nebula is so large that some parts of it should be radiating slightly circularly polarized light, according to this model, and it would seem that further observations to test for a few per cent circular polarization in the north-west and south-east parts of the nebula will very soon resolve the viability of the model one way or the other.

In this issue of *Nature* (p. 103), Landstreet and Angel report observations of the optical polarization of the light from the Crab Nebula which are of crucial importance in distinguishing the mechanisms active in the source. This work certainly must leave a question mark against the theory at present.

## PLATE TECTONICS

**On the Move**

from a Correspondent

A MEETING held on February 25 at the Royal Society to discuss plate tectonics and the evolution of the Earth's crust turned out to be both lively and controversial. Dr D. P. McKenzie (University of Cambridge) began by explaining that the original plan of the organizers had been to hold a meeting about the geodynamics project, but had changed their minds in favour of a discussion of the scientific problems with which the project is concerned. He then went on to talk about the present day deformation of the Mediterranean, which is dominated by two small, rapidly moving fragments of continental plate, and warned that similar continental plates might have existed in other older mountain belts.

Dr N. Ambraseys (Imperial College, London) then discussed the historical records of the seismicity of the eastern Mediterranean, which clearly showed that the same belts were active in the past as are now active. But some regions such as south-eastern Turkey have had few large recent earthquakes compared with the number expected from the old records, and therefore recent activity does not give a good indication of the long term seismic risk, although it is generally sufficient to define the active plate boundaries. Dr A. G. Smith (Sedgwick Museum, Cambridge) and Professor K. Hsü (Ecole Polytechnique Fédérale, Zurich) both talked about their attempts to reconstruct the arrangement of the continental fragments within and around the Mediterranean basin in the lower Jurassic. Both speakers were impressed by the absence of deep oceanic sediments in the rocks around the western Mediterranean, and argued that the western Mediterranean is not a relic of Tethys but has been formed by sea floor spreading since the lower Jurassic. Although Smith and Hsü agreed on the general outline, the details of the reconstructions they presented were different and neither believed that there was yet enough information to produce an accurate map.

The two major questions which were not discussed in the early contributions on plate tectonics were the nature of the driving mechanism and the extent to which ancient continental tectonics can be discussed using the concept of rigid caps in relative motion. Professor J. Sutton (Imperial College, London) began the afternoon by arguing that the Precambrian tectonics of the greenstone belts seems to be quite different from that of present day tectonics, and illustrated this point with some remarkable slides of these old belts. He and later speakers agreed that the change from

the greenstone belt type of tectonics, with its linear belts of basic volcanics separated by large circular granite and gneiss domes, occurred about  $2.7 \times 10^9$  years ago, and remarked that active and inactive regions could be clearly recognized in rocks  $1.8 \times 10^9$  years old. Drs C. J. Talbot (University of Dundee) and B. F. Windley (University of Leicester) discussed various mechanisms by which the greenstone belts could have been formed, but both emphasized how incomplete is our present understanding of their origin.

The other major problem concerns the driving mechanism. Dr L. Lliboutry (University of Grenoble) and Professor S. K. Runcorn (University of Newcastle upon Tyne) both put forward possible mechanisms which were radically different, and the discussion after their contributions showed that there was still no general agreement on the nature of the convection which moves the plates. This seems to be the field in which least progress has occurred in the past few years. Dr X. T. Le Pichon (Centre

Océanographique de Bretagne, Brest) put forward the view that a detailed study of the history of the relative motion between major plates may help our understanding of this problem.

Few related disciplines have so far made any use of the concepts of plate tectonics. In particular, the distribution of animals, plants, palaeoclimatology and palaeoecology must be closely connected with the evolution of continents and ocean basins. Dr P. L. Robinson (University College, London) suggested various simple rules by which the climate of a region could be obtained from a continental reconstruction, but pointed out that our present understanding of oceanic and atmospheric circulation was not yet sufficient to make detailed predictions.

The meeting showed that plate tectonics has suggested many new lines of research in related subjects, but also demonstrated that the new theory still has several major difficulties to be overcome before it is the theory of global tectonics.

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**Are Gravity-Geomagnetism Correlations Valid?**

THE hypothesis that the interface of the Earth's core-mantle is not perfectly smooth but undulates has been neither confirmed nor refuted directly. For example, there is no evidence of topography from the travel times of compressional waves reflected at the interface; but this only means that if undulations are present their height must be less than a few tens of kilometres, the present lower limit of resolution of seismological methods. It is quite possible that the resolution may be improved by the use of seismometer arrays and better methods of data analysis; and so the search for evidence of topography from PcP waves is still going on. In the meantime, however, evidence must be sought by indirect methods—by predicting the consequences of interface topography of the core-mantle and then showing that such consequences obtain.

The correlations between global features of the Earth's magnetic field and gravitational field recently found by Hide and Malin (*Nature*, **225**, 605; 1970) could be taken as just such evidence, though other explanations are possible. If undulations are present at the core-mantle interface there will be density variations which may produce large-scale gravitational anomalies at the Earth's surface. At the same time such a topography might interact with the magnetohydrodynamic motions which produce the magnetic field in the Earth's core, especially the non-dipole field which is thought to originate in the vicinity of the core-mantle boundary. What Hide and Malin showed was that there is indeed a sig-

nificant correlation between the Earth's gravitational field and the non-dipole part of the geomagnetic field as long as the latter is displaced eastwards by about  $160^\circ$  longitude. The correlation coefficient for this is about 0.84 which, according to Hide and Malin, is so high that the odds against its occurring by chance are more than one hundred to one.

But in next Monday's *Nature Physical Science*, Lowes and Khan independently take issue with Hide and Malin on the supposed significance of the correlations. Lowes's essential point is that the odds against the correlations is not one per cent, as Hide and Malin suggest, but rather between 5 and 10 per cent. Thus he does not seem to doubt the existence of the basic correlations but suggests that such a high probability of the correlations occurring by chance must throw doubt upon their physical significance. He also completely rejects the significance of the correlations between undisplaced gravitational and magnetic fields recently found by Khan and Woollard (*Nature*, **226**, 340; 1970). Khan, on the other hand, has devised a modified test which purports to show that all gravitational-magnetic correlations are insignificant.

In their reply, which follows the original criticisms, Hide and Malin reject Khan's point completely on the grounds that an analytical error in his test invalidates it. By devising other significance tests they also beg to differ from Lowes. In other words, Hide and Malin are quite prepared to stick to their conviction that their original correlations are valid.



## RNA POLYMERASE

**Multiple Forms**

from our Cell Biology Correspondent

BACTERIA, no doubt because of evolutionary pressures, are remarkably economical housekeepers. It is far from easy, for example, to think of ways of improving on nature when the problem is one of providing a unicellular organism with the ability to switch rapidly from the synthesis of one set of protein and RNA molecules to the synthesis of another set at the dictate of the environment. Having basic machinery which has the capacity to catalyse the synthesis of peptide or phosphodiester bonds but lacks the specificity required to decide which particular proteins or RNAs to make until it is programmed by a messenger RNA or a sigma factor is probably the ideal solution. Obviously, it takes far less time and energy to synthesize new programming molecules than to synthesize complete new sets of hardware.

Eukaryotes have during the course of evolution retained this flexible and economic mechanism of protein synthesis and the betting must surely be that they also programme their RNA polymerases with sigma factors. But, equally, it is becoming increasingly clear that in the nuclei of eukaryotic cells there are at least two and possibly, in some if not all cells, three distinct forms of RNA polymerase each of which presumably has some degree of built-in specificity for particular DNA sequences. During the past two years there have been so many reports of two separable RNA polymerases in a range of animal and plant cell nuclei that few cell biologists now question the generality of this finding. Until now, however, three RNA polymerases have only been isolated from one material, developing sea urchins, but in the current *Proceedings of the US National Academy of Sciences* (68, 338; 1971) Horgen and Griffin report another example of this situation. They have detected three RNA polymerases in extracts of nuclei isolated from the aquatic fungus *Blastocladiella emersonii*; these three activities can be distinguished not only by their behaviour on chromatography but also with specific inhibitors.

Two of the three polymerase activities resolved by passing extracts of *Blastocladiella* nuclei through columns of DEAE-cellulose seem homologous to the two activities found in all the eukaryotic nuclei examined to date. One (polymerase II) is sensitive to alpha-amanitin, and like comparable enzymes in other cell-types it no doubt resides in the nucleoplasm where it is probably involved in the synthesis of messenger and other non-ribosomal

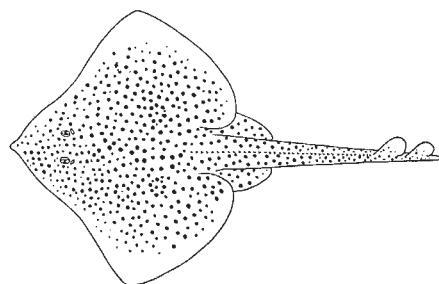
RNAs. The second activity (polymerase I), which like its counterparts in other cells is located in the nucleoli (where it probably transcribes ribosomal RNA cistrons), can be specifically inhibited *in vitro* by cycloheximide, albeit at concentrations far in excess of those required to inhibit protein synthesis. The third polymerase fraction by Horgen and Griffin resembles the RNA polymerase of bacteria in one important respect: it is inhibited by rifampicin. Of course, this enzyme may come from mitochondria which might have contaminated the nuclei isolated from the *Blastocladiella* cells—it should be possible to test rigorously this suggestion. Alternatively, this third enzyme may be an authentic nuclear polymerase, which, like a vestigial organ, points to the evolutionary ancestry of the fungus.

## SKATES

**Taxonomic Revisions**

from our Marine Vertebrate Correspondent

AMONG the cartilaginous fishes the rays and skates have developed a near monopoly of life on the sea floor. Beautifully adapted to exploit a largely two dimensional environment they



Spotted ray, *Raja montagui*, found in European waters.

have colonized many tropical fresh waters, the deep sea, and the shallow seas from the Arctic Circle to the Antarctic. With such a wide distribution it is not surprising that very many species have been described and the taxonomy and classification of the group present a very confused situation. Even among the family Rajidae in the north-eastern Atlantic, which includes the moderately important commercial skates, there are many taxonomic problems.

A recent revision of the European skates by M. Stehmann (*Arch. Fisch. Wiss.*, 21, 73; 1970) goes some way to solving some of these problems. Much of the earlier confusion in the

**Explosive Nucleosynthesis in Supernovae**

SINCE the classic article by Burbidge, Burbidge, Fowler and Hoyle in 1957, it has been generally agreed that the heavy elements were produced by nuclear reactions in stars. Initially, the nuclear processes seemed fairly well understood but their association with particular types of stars was less clear. Recently, work by Arnett and his collaborators has suggested that the relative abundances of essentially all nuclei between neon and nickel can be explained by explosive burning of carbon, oxygen and silicon in supernovae. It is desirable to test these theoretical predictions concerning supernova explosion in two ways. First, are the properties of observed supernovae similar to those predicted by theory and, second, can the total abundances of the moderately heavy element be explained by an acceptable number of supernova explosions?

In next Monday's *Nature Physical Science*, M. V. Delano reports his studies of the second question. He estimates that the mass of the moderately heavy elements and unburnt lighter elements which must have been expelled by supernovae is of the order of  $3.5 \times 10^9$  solar masses. The best estimate of the current rate of supernova explosions in galaxies similar to our own is about one every 23 years. If the supernova rate in the galactic life of about  $10^{10}$  years has never exceeded that value, an average of 8 solar masses must have been expelled in each explosion. Observations that only the oldest stars

are very deficient in heavy elements indicate, however, that a large fraction of these elements were produced early in the galactic history so that the supernova rate was probably much higher then and the mass expelled by each supernova can have been correspondingly less.

Delano has considered a variety of possible past rates of supernovae and values of supernova masses and ejected mass. He concludes that the best agreement between theoretical supernova models and the total production of heavy elements is based on the assumption that supernovae of about 5 solar masses exploded at a rate of about 10 per year for the first few per cent of the galactic lifetime and that there has been a relatively steady and much lower rate since then.

Such a description may be correct, but there are still unsolved problems concerned with the comparison of theoretical and observed supernovae. Very few supernovae have been studied in detail but the estimates of ejected masses are generally lower than the amount required for nucleosynthesis and there is no clear evidence that supernova debris is excessively rich in heavy elements. In addition, some theoretical calculations produce objects which expel masses of process material but never become very luminous. Invisible supernovae may play an important part in nucleosynthesis. Alternatively, explosions in galactic nuclei may be a significant source of heavy elements.

group has arisen from an over reliance on external morphological features, in a group in which there are considerable differences between the sexes, and between the young and mature fish of most species. Stehmann's treatment of the problems shows a welcome advance in that he has used internal as well as external morphology. Three characters, in particular, have proved of value: the shape of the cranium, the development of the rostral cartilages and, in males, the anatomy of the intromittent organs.

Using these characters Stehmann finds that the European rays fall into a number of clear taxonomic groups, and this division is supported by other external features. His conclusion is that twenty-two species in the area can be divided into two genera, all but three in the well known genus *Raja*. In his classification, however, *Raja* is broken down into six subgenera (two of which he names for the first time). This splitting brings problems in its wake, for five species are not placed positively in any of these pigeonholes, and may, according to the author, actually represent another three unnamed subgenera. In view of these unsettled problems it is doubtful whether this division into numerous subgenera will be followed by other workers.

Stehmann's paper was just preceded and conveniently supplemented by a revisionary investigation of the Rajidae of the west and south coasts of southern Africa by P. A. Hulley (*Ann. S. Afric. Mus.*, **55**, 151; 1970). Hulley confirms that the skate fauna of southern Africa has several species in common with Europe. It is, however, much less well known and he describes five new species, three of them from deep water. This investigation also relies on the structure of the claspers of adult males, as well as the rostral cartilages. Hulley is conservative in his treatment of the taxonomy of Rajidae and he recognizes only three genera—*Cruriraja* (three species), *Bathyraja* (one species) and *Raja* with eighteen species—although his study shows natural relationships between the species and suggests that regrouping of the genus *Raja* at subgeneric or generic level would be justified. This Hulley proposes to examine in a later article.

## VIROLOGY

### Vertical Transmission

from our Cell Biology Correspondent

THE vertical transmission of viruses or viral genomes from parents to offspring by way of the sperm and/or egg, rather than the familiar horizontal transmission by infection, is a topic of increasing importance these days, especially in the field of tumour virology. For, quite apart from any

speculations about oncogenes, although there is ample evidence proving that at least in certain species of animals cancers can be caused by viruses, there is little evidence to suggest that most cancers are infectious diseases. This paradox is, of course, simply resolved by postulating that cancer viruses can be transmitted vertically.

No doubt this line of reasoning is at the bottom of the current work of Koprowski and his colleagues. They have recently reported that mammalian ova can be infected with the small DNA tumour viruses, and in the current issue of the *Proceedings of the US National Academy of Sciences* (**68**, 353; 1971) Brackett, Baranska, Sawicki and Koprowski report that rabbit sperm can not only take up the DNA of simian virus 40 but also transfer it, in an infectious form, to ova at fertilization.

Autoradiographs of rabbit spermatozoa exposed to SV40 virus containing DNA labelled with  $^3\text{H}$ -thymidine indicate that the virus particles fail to penetrate these cells. The same conclusion stems from experiments in which rabbit spermatozoa were exposed to SV40 and then fused, with inactivated Sendai virus, into African green monkey cells either directly or after treatment with anti-SV40 serum. A cytopathic effect could be seen in the monkey cells (which support the complete replication of SV40) receiving the

sperm treated with the virus but not the antiserum within 72 hours of the fusion. The cytopathic effect in cells receiving the sperm treated with antiserum as well as virus was by contrast much delayed or not detected. Clearly, SV40 sticks to the surface of rabbit sperm but fails to penetrate these cells.

On the other hand, autoradiographs of spermatozoa exposed to naked SV40 DNA labelled with  $^3\text{H}$ -thymidine revealed the viral DNA in the post-acrosomal region of the sperm and in one experiment some 16 per cent of the input viral DNA was recovered with the sperm DNA; apparently, SV40 DNA is taken up by rabbit sperm. That this incorporated viral DNA is infectious was shown by fusing the sperm with African green monkey cells; cultures of the heterokaryons eventually yielded infectious SV40 virus.

But does the viral DNA survive in a fertilized ovum? To answer this question, Koprowski's group fertilized rabbit ova with rabbit sperm which had taken up SV40 DNA. The fertilized eggs were then plated on monolayers of the permissive green monkey cells and cultured. In nine out of twenty-three such cultures, monkey cells showing a cytopathic effect were seen and the fluids from these cultures were shown to contain SV40. The viral DNA had been transferred to the eggs at fertilization and at least some of it remained infectious.

## Length-slow Chalcedony and Evaporites

EVAPORITES are rocks that form by crystallization from land-locked saline waters as they evaporate in warm dry climatic conditions, and their principal constituent minerals are anhydrite ( $\text{CaSO}_4$ ), gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), and halite ( $\text{NaCl}$ ). Hence, when evaporites form part of a rock sequence, they are of use in inferring past climatic and geographic conditions. But because the constituent minerals are soluble, they are easily removed from rocks at a later stage by percolating water so that there remains little or no trace of their former presence. A reliable means of identifying missing evaporites would not only provide evidence of ancient geography and climate, and data relating to theories of continental drift, but would also help to augment and interpret the stratigraphic record.

Chalcedony is a fibrous form of silica deposited from aqueous solutions, and the fibres are usually described as length-fast (that is to say, the refractive index along a fibre is less than that across the fibre). It has long been known, however, that the fibres are sometimes length-slow; the distinction between the two types is easily made by colour with the aid of a so called sensitive tint plate when thin sections are examined between crossed

polars with the polarizing microscope.

J. S. Pittman and Robert L. Folk report in next Monday's *Nature Physical Science* that they have discovered that the less common length-slow variety of chalcedony seems always to be associated with evaporites, either in the evaporite itself or replacing carbonate minerals (for example, in fossils) in adjacent rocks. Careful study of sections containing length-slow chalcedony has in many cases revealed unsuspected inclusions of relict evaporitic sulphate minerals, or textures characteristic of evaporites, giving strong support to the hypothesis. The beauty of this discovery is the ease with which it may be applied, obviating the need for tedious scrutiny of rocks in which the chalcedony is length-fast.

Pittman and Folk give no account of the mechanism by which chalcedony is produced; nor, indeed, is it known why the fibres of common chalcedony should be length-fast when larger crystals of quartz are always length-slow. It is well known that the shape of crystals may be influenced by the presence of "foreign" atoms in the solution from which they crystallize, and in the case of chalcedony calcium sulphate may prove to be a controlling factor.



## PROTEINS

**Mercurial Enzyme**

from our Molecular Biology Correspondent

AMONG the many recent examples of survival of enzymes in working condition in face of the most extreme forms of chemical violence and abuse, none is more astonishing than that just described by Sperling and Steinberg (*J. Biol. Chem.*, **246**, 715; 1971). Earlier work from the same laboratory had shown that considerable liberties could be taken with the disulphide bonds of pancreatic ribonuclease without complete annihilation of activity. On the one hand, two of the four bonds could be reduced, leaving an active product and, on the other, one disulphide bond could be reduced and a mercury atom inserted, also with no untoward consequences.

Sperling and Steinberg now describe the insertion of mercury into all four bonds. Mercuric ions will react with thiols to form the group  $-S-Hg-S-$ , this being 3 Å longer than  $-S-S-$ . When mercuric chloride is added to reduced ribonuclease in dilute solution a product, homogeneous by all criteria and sedimenting at the rate associated with the native enzyme, is generated, which contains four atoms of mercury per molecule of protein. This material has 25 per cent activity towards cyclic CMP and 5 per cent towards RNA. Sperling and Steinberg have gone to all necessary lengths to demonstrate that the activity is a property of the modified ribonuclease and is not associated with a contaminating fraction of reoxidized enzyme. In the first place the material migrates as a single zone in gel electrophoresis, but with a mobility different from that of native ribonuclease. Second, it is formed at pH 4.6, where disulphide bonds do not re-form and reduced enzymes do not renature. Third, the modified ribonuclease, unlike the native, is digested by trypsin at a moderately rapid rate (though more slowly than the unfolded oxidized protein); the loss of enzymatic activity parallels the rate of digestion, and cannot therefore be due to a contaminant of the resistant native enzyme.

In addition to the proteolytic lability, the mercury complex is substantially devoid of the Cotton effect seen in circular dichroism in the region of tyrosine absorption. The environment of one tyrosine is therefore appreciably different, which is consistent with the further observation that one of the three un-titratable tyrosines of the native enzyme is normalized in the modified form. Not only then is the mercury sufficient to induce correct refolding in spite of the 3 Å stretch that it causes, but the structure can also absorb this very large distortion with little enough discomfort that activity is preserved.

A key to this phenomenon may perhaps be found in the analysis by Lee and Richards (*J. Mol. Biol.*, **55**, 379; 1971) of side chain packing in known globular protein structures. In ribonuclease they find that there are three cavities, one of them of considerable volume (calculated as 0.375 Å). This may be important in absorbing some of the slack generated by intruding elements, such as the four mercury atoms.

From an artificial to a natural change of geometry—Bustin and Conway-Jacobs (*J. Biol. Chem.*, **246**, 615; 1971) have re-examined the activation process in pepsinogen. As with other zymogens activation is triggered by a specific proteolytic cleavage of the chain. This much was established more than thirty years ago, but more recently evidence of more complex interrelations has accrued, for the nature of the reaction depends on the pH. In order to follow the appearance of proteolytic activity pepsinogen was added at acid pH to a protein substrate, haemoglobin, and the rate of digestion followed. When partly predigested haemoglobin was used proteolysis

began without a lag, and the specific activity was independent of pepsinogen concentration, and was not increased by added pepsin. This strongly suggests that the pepsinogen is self-activating under these conditions. This inference was supported by the elegant conceit of binding the pepsinogen covalently to a 'Sephacrose' matrix, so that all the molecules are isolated from each other, and showing that at pH 2 the zymogen in this state underwent rapid activation. In these circumstances it is scarcely possible that traces of pepsin are involved, or that the reaction is intermolecular. The internal cleavage to expose a second N-terminal group could be followed in the matrix-bound protein. At acid pH the conformation evidently changes in such a manner as to create an active site, which forthwith bites another part of its own chain. Whether the haemoglobin substrate is digested by pepsinogen or pepsin is not revealed. This is the second example of a self-activating zymogen, the other being that of streptococcal protease, described last year by Bustin, Lim, Stein and Moore.

**Cooperating Cancer Viruses**

It has been suggested that it might be possible to screen for individuals and families running an abnormally high risk from cancer by measuring the susceptibility of human cells to transformation by the DNA tumour virus SV40. The idea stems from the discovery that cells taken from patients with such diseases as Down's syndrome, Klinefelter's syndrome and Franconi's anaemia—diseases associated with a high incidence of cancer—and from a family with a history of multiple sarcomas, are all markedly more susceptible to transformation by SV40 than the cells of normal people. In next week's *Nature New Biology*, Rhim and his colleagues report experiments which may have some bearing on these observations. They have found that rat embryo cells infected with the Rauscher strain of mouse leukaemia virus are more efficiently transformed by SV40 than comparable uninfected rat embryo cells. It is not inconceivable that these rat cells infected by murine leukaemia virus and human cells from people who run a high risk of developing cancer have certain properties in common which render them unusually susceptible to transformation by SV40.

The established lines of rat embryo cells infected by the Rauscher leukaemia virus, which Rhim *et al.* selected, had been producing progeny virus particles for more than 18 months whereas parallel lines of uninfected cells neither contained

leukaemia virus antigens nor produced the virus itself. Cultures of the two classes of cells were exposed to SV40 at the same multiplicities of infection, subcultured after a week and then allowed to grow for a further two weeks. By that time foci of transformed cells could easily be distinguished, because within the foci the cells grew to several layers deep. There were up to about ten-fold more foci of transformed cells in cultures of the cells infected with leukaemia virus than in the corresponding uninfected cultures. Moreover, the titres of SV40 induced tumour antigen were also much higher in the former than in the latter cultures.

Clearly, rat embryo cells infected with murine leukaemia virus are more susceptible to transformation by the DNA tumour virus SV40 than comparable cells which are not infected by the leukaemia agent. This is the first example of infection by an RNA tumour virus enhancing a cell's susceptibility to transformation by a DNA virus and the molecular basis of the phenomenon has yet to be deciphered. For example, it might be that the cells infected with the leukaemia virus more efficiently take up or uncoat SV40, or perhaps there are other intracellular changes in these cells which promote some later step in transformation by SV40. It will, however, be of great interest, and possibly of relevance to human cancer, if the former possibility proves to be the case.

## REPTILIA

**Galloping Crocodiles**

from our Vertebrate Palaeontology

Correspondent

EVERYBODY knows that crocodiles are amphibious reptiles, and many know that they are the closest living relatives of the dinosaurs. It now seems, however, that the early stages of dinosaur evolution included forms which were very unlike a modern crocodile in appearance and way of life. This has become clear from a revision of the Jurassic reptile *Hallopus* by Dr Alick Walker, of the Department of Geology, University of Newcastle upon Tyne (*Phil. Trans. Roy. Soc. Lond., B*, **257**, 323; 1970).

The single known specimen of *Hallopus* was last examined nearly 60 years ago. As always, the lapse of time has been accompanied by new techniques of preparation (and by higher standards of preparation), and by new discoveries of related Mesozoic reptiles. Walker has therefore been able to revise several features of *Hallopus* and to reveal their true significance.

*Hallopus* had previously been considered as a rather primitive coelurosaur — themselves the most primitive of the saurischian dinosaurs. The skull, which would of course have provided the clearest indication of its affinities, is unfortunately missing. *Hallopus* had been thought to be specialized in having a very short fore limb, but Walker has found that this opinion arose from several misidentifications of bones by earlier workers. In fact, the notable feature of the fore limb is the elongation of the bones of the wrist (radiale and ulnare), and it is also significant that the ulna extends distally beyond the radius. Both these features are uniquely crocodilian, though the degree of elongation of the wrist in *Hallopus* is exceptional. The ankle of crocodiles is equally distinctive: there is a peg-and-socket joint within the ankle, one bone of which is functionally part of the foot, whereas the other is immovably united with the lower part of the leg itself. Here again, the osteology of *Hallopus* is basically crocodilian, but with an unusual modification, for the tibia is elongated and the foot is functionally tridactyl.

Further analysis of *Hallopus* alone would be difficult, because the specimen is so incomplete, but Walker is able to show that it is descended from a group of Upper Triassic genera whose affinity with one another had not previously been recognized. This group, which he places within the Crocodylia and names the Pedeticosauria, similarly shows a tendency towards elongation of the limbs, though this is not as extreme as in *Hallopus*. Walker believes that the

hind foot of *Hallopus* was digitigrade, and that its hind limb as a whole indicates a running, cursorial mode of life. This seems also to have been true of some, at least, of the Triassic pedeticosaurids.

Not all the Triassic crocodiles were pedeticosaurids, but even the others (for which Walker uses the term Protosuchia) show indications of cursorial tendencies. This is, at first sight, unexpected in the ancestry of the living crocodiles. Walker recalls, however, Cott's observation that young Nile crocodiles can gallop, "bounding along like a squirrel", and suggests that this gallop may preserve a very ancient type of crocodilian locomotion. This would explain the loss of the clavicles, for this is a feature which is found in many cursorial groups; by freeing the shoulder

girdle from the rib cage, this allows the shock of landing to be absorbed by the musculature. Indeed, Walker plausibly argues that a galloping habit may have been a pre-adaptation for amphibious life. Adaptations for galloping may have been the original cause for several features, such as the elongation of the coracoid and resulting more lateral position of the shoulder joint. These features could have been equally advantageous in amphibious life, allowing greater efficiency of the limb in swimming, steering, slithering and "fending-off" movements. The change from terrestrial to amphibious life was presumably because even a galloping crocodile was no match for the large carnivorous dinosaurs which became the dominant terrestrial reptiles in the Jurassic.

**New Bacteriophage DNA Transcriptase**

IN next Wednesday's *Nature New Biology*, Dunn, Bautz and Bautz report the isolation and partial characterization of a DNA dependent RNA polymerase, or transcriptase, specified by the bacteriophage T3. This enzyme is in many respects identical to the transcriptase specified by the closely related phage T7, but in spite of their close similarities these two enzymes transcribe their homologous DNA far more efficiently than they transcribe each other's DNA.

Less than a year ago it was widely believed that when the phage T7 infects an *Escherichia coli* cell, the phage genome specified a new sigma factor, which, by combining with the host cell's transcriptase, subverted that enzyme to the transcription of the phage DNA rather than the bacterial DNA. That is the sequence of events during the replication of bacteriophage T4 and there seemed every reason to expect that new sigma factors were the answer to every problem of changing the specificity of transcription in bacterial cells infected with phages. But last autumn, Chamberlin and his colleagues put the cat among the pigeons by proving unambiguously that among the first four genes transcribed when T7 infects *E. coli* is one which specifies a completely new enzyme, rather than a new sigma factor. This T7 transcriptase transcribes the late T7 genes and allows the replication of the phage to be completed. At the same time the transcription of the first four T7 genes and the bacterial genes ceases, which suggests that some other T7 gene product somehow inactivates the host cell transcriptase once it has provided the phage with its own specific enzyme.

Bacteriophage T3 is a very close relative of T7 and, for once, things are as they might be expected to be. T3

specifies its own transcriptase which, in its insensitivity to antibiotics such as rifampicin and streptolydigin, in its failure to utilize  $Mn^{2+}$  in place of  $Mg^{2+}$  and in its sensitivity to increasing ionic strength, closely resembles the T7 enzyme. The two enzymes can, however, as Dunn and the Bautzes show, readily be distinguished by their capacity to transcribe either T3 or T7 DNA. Each enzyme is markedly more active when it is presented with its own DNA than when it has the heterologous DNA as template.

It seems that either enzyme can bind efficiently to either DNA but the initiation of the synthesis of an RNA chain is only efficient if the enzyme is bound to its own DNA. This suggests, of course, that the promoter sequences which tell the enzyme to start making RNA differ slightly in the two sorts of DNA. This, and the fact that both the T3 and T7 enzymes are single polypeptide chains weighing about 110,000 daltons, is most important to biochemists bent on finding out precisely how RNA is made off DNA templates. The transcriptase of *E. coli* is made up of five polypeptide chains and such complexity does not facilitate studies of the precise interactions between the enzyme, its template and its product. By comparison the two phage enzymes are simple; furthermore, temperature sensitive and nonsense mutants are already available and no doubt more can be obtained to order. From these it should be possible to obtain transcriptases with altered specificities which should greatly facilitate detailed analyses of just how this enzyme recognizes certain base sequences and responds to them by making RNA. Clearly, much more will be heard about the DNA dependent RNA polymerases of T3 and T7.



## LOCOMOTION

**Stepping and Jumping**

It is not really surprising that very few zoologists have investigated the gait of kangaroos but this situation has now been remedied by D. E. Windsor and A. I. Dagg of the University of Guelph in Canada. These authors have not only identified four types of gait in the Macropodinae, the marsupial subfamily which includes kangaroos and wallabies, but have also used this information to reconstruct the phylogeny of the group (*J. Zool.*, **163**, 165; 1971).

For their study, Windsor and Dagg took 360 m of film of nineteen species of macropod at seven zoos. Where possible, they placed stakes at measured intervals along each runway or they measured distances between fence supports so that they could calculate the speeds of the animals. This method was more accurate than measuring the locomotion of wild macropods in their natural habitats where the ground is often uneven and vegetation obscures movements. The four gaits identified were a slow progression, a walk, a quadrupedal bound and a bipedal hop.

The slow progression involves all the limbs plus the tail and is used chiefly while the animal is grazing. There were no significant differences between any of the species studied. This is to be expected, the authors say, because the centre of gravity of members of this group lies behind the hindfeet; thus the macropod has a triangle of support at the beginning and at the end of each stride and only during one part of the stride is the animal supported by two appendages alone.

The walk is the only gait in which the pairs of limbs are not used synchronously and is confined to the arboreal genus *Dendrolagus*, the tree kangaroo. This gait is similar to the common quadrupedal walk of most mammals. Without it, *Dendrolagus* would be restricted to branches which were sufficiently thick to permit the simultaneous use of the forelimbs or hindlimbs as in the slow progression.

The quadrupedal bound is considered to be a primitive gait because it is only found in the short tailed wallaby (*Setonix brachyurus*) and the tree kangaroos *Dendrolagus matschiei* and *D. goodfellowi*, which are believed to be close to the primitive phalangerid ancestors of macropods. This gait involves the use of the hindfeet and then the forefeet in sequence and it was found by Windsor and Dagg to be statistically similar in the three species which move in this way. Windsor and Dagg suggest that the ancestors of the present Macropodinae used this gait for movements faster than slow progression would allow. Because the ancestral habitat was rain forest, quad-

rupedal gait would have allowed the primitive macropod to change direction as it dodged among the obstacles on the ground. The gait intergrades with the bipedal hop; it seems likely therefore that as rain forest gave way to open terrain and plains, some of the developing macropods took to using the faster bipedal locomotion and eventually the slower quadrupedal gait lost ground completely to the bipedal hop in the more highly developed plain dwelling species of Macropodinae. Faster gait was not necessarily a selective advantage in these large kangaroos because they evolved in an environment with few serious predators. The locomotory adaptation of large macropods in open terrain seems therefore to be for quick escape from danger rather than for maintaining high speeds as a means of outrunning predators.

Windsor and Dagg found that the pattern of bipedal hop varies considerably with the habitat of the species. Some use their hindfeet for a relatively long proportion of the stride, and some for a relatively short proportion of the stride. Species such as the wallaroo or euro, *Macropus robustus*, which live on rocky hills have increased suspension which allows them to mount steep

slopes and leap among rocks. In contrast, the red kangaroo, *Macropus rufus*, which lives in open plains areas has a shorter period of suspension.

From their measurements, Windsor and Dagg draw several conclusions about the evolution of the Macropodinae and they have constructed a phylogenetic tree. The characteristics of the slow progression of the brush wallaby, *Wallabia bicolor*, suggest that it is closely related to the short tailed scrub wallaby (*Setonix brachyurus*), the New Guinean forest wallaby (*Dorcopsis veterum*), and the tree kangaroo *Dendrolagus matschiei*; but it is probably less primitive than *Setonix* and *Dendrolagus* because it does not seem to show quadrupedal bound. Windsor and Dagg support earlier work which favours removing all the species of *Wallabia* except *W. bicolor* to the genus *Macropus*, leaving the genus *Wallabia* as monotypic. Turning to *Macropus*, Windsor and Dagg say that the bipedal hop gait of the red kangaroo, *M. rufus*, is significantly different from that of *M. robustus* and *M. melanops*. This, they say, confirms previous suggestions (supported by chromosome counts) that *M. rufus* should be placed in a separate genus, *Megaleia*.

**Water Abundance Weakens Apical Control**

IN next Wednesday's *Nature New Biology*, fresh light is cast on the thorny botanical problem of how the apex of a growing plant controls the growth and development of the lateral buds farther down the stem. G. I. McIntyre of Regina Research Station, Saskatchewan, suggests that the supply of water to the lateral buds may play a much greater part in the phenomenon of apical dominance than had previously been thought. His results indicate that when water is in short supply, the apex exerts complete control over the lateral buds and they show almost no growth. When water is freely available, however, this control is by no means so complete; when the relative turgidity of the leaves exceeds 80 per cent, the axillary buds are, to a large extent, released from apical control.

Apical dominance is the most obvious of the growth correlations shown by plants, those phenomena in which the growth of one region of the plant interacts with, controls or inhibits the growth of other parts of the plant. As such, apical dominance has been studied intensively, both for its own sake and in the hope of unearthing clues to the nature of the less overt correlation mechanisms. Nevertheless, the phenomenon has yielded few of its secrets.

Briefly, extension growth in plants stems is confined chiefly to the apical regions of the main shoot. Lateral buds are formed at regular intervals down the

main stem, but the further growth of these buds is inhibited; they are stimulated to develop further only if the shoot apex is destroyed, or if the control which the apex exerts over the laterals is broken in some other way.

Two principal explanations for this control are currently in vogue. The first holds that the buds are prevented from developing by a plant growth hormone or combination of plant growth hormones derived from the dominant apex and transmitted towards the base of the stem. The second theory is rather more controversial and is based on the idea of hormone-directed transport, a development of the nutrient diversion theory. This idea was formulated about thirty years ago and is based on the proved observation that nutrients flow preferentially towards regions of high hormone concentration. In other words, a more sophisticated version of the old "nutritive" theory, popular around the turn of the century.

McIntyre's theory could be interpreted as an extension of this latter idea, although he has no evidence to suggest what mechanism may direct the flow of water through the plant principally towards the dominant apex. But as he shows clearly that when water is in abundance, carbohydrate or nitrate availability is the limiting factor in breaking apical dominance, it is likely that some hormonal mechanism is responsible.

# Brain Drain from Developing Countries

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**If the loss of scientific and technical manpower from the developing countries is to be stemmed, firm measures must be taken soon. These suggestions for tackling the problem are a modified version of a paper read at the Pugwash conference on science and world affairs in October 1969. The views expressed here are, of course, those of Dr Parthasarathi and do not reflect the views of the Government of India.**

THERE are several reasons for developing countries to continue to be concerned about their loss of scientific and technical manpower through migration. First, there is increasing evidence that some developing countries are losing so much of their manpower that their capacity to tackle their social, economic and cultural problems is being damaged irreparably. Second, much international cooperation is based on the idea that the high income countries should be supporters rather than prime movers. But this can be a meaningful basis for aid only if developing countries possess their own primary scientific resources. The magnitude and quality of the talent that many developing countries will lose if present trends continue, however, raise serious doubts about the generation and growth of sufficient endogenous scientific and technological activity for any external action to be of much use. Third, the response of the high income countries to this problem is an excellent indication of whether they are prepared to place commitment to world wide development above short term national self-interest.

My suggestions for policies and action which can and must be taken now on a unilateral, bilateral and multilateral basis, are based on at least two premises. First, statistics of the emigration of scientific and technical personnel from low to high income countries, and the composition and dynamics of such emigration during the past eight years, indicate that some categories of manpower at least constitute a loss to the country concerned. Second, there is evidence that the total outflow of students, trainees and "fully trained manpower" from low to high income countries is increasing rapidly.

## Responsibilities at Home

Certain initiatives should be taken by the developing country itself. The Government of India, for example, has progressively reduced the range of disciplines and areas of specialization for which an Indian can go abroad to study. In such cases, before permission is given, students must show that they have reached the highest level of training

available in India. It should, however, be a general policy that no overseas study is allowed until the student or trainee has been educated to the highest possible level in his own country. After this every student or trainee should be employed at home for at least two years, before being permitted to study abroad. Such a requirement, which of course needs legal sanction, must also be backed by several governmental and non-governmental measures to make use of the students.

Available information about apprentice training programmes in India suggests that private industry tries to avoid taking its share of the social cost of such training. Governments would therefore have to take such measures as instituting industrial fellowship schemes for supporting engineering manpower in industry for the two years. Similar support would have to be provided in medical and other government services, and also in major research and development establishments. Such a procedure might, in an appropriate political and economic environment, familiarize the young scientist, engineer or doctor with real developmental tasks. This experience could have a powerful effect on the discipline or area that he chooses for further study abroad (at the end of the two years) or may even lead to a re-evaluation of the costs and benefits of overseas study.

It is appropriate and necessary to ask whether developing countries, such as India and the United Arab Republic, can continue to run a "mixed economy" in their manpower development programme. Is it meaningful to bring domestic higher education within the purview of planning, while leaving overseas training to the "international manpower market"? It should be remembered that the forces determining the dynamics of that market are beyond the control of the developing countries concerned. It seems, therefore, that it will be impossible to avoid the setting of quotas for overseas training in terms of countries, type of degree/training programme, sub-disciplines and the intellectual attainment of candidates, if meaningful manpower planning is to be undertaken. There have been precedents for this approach, from Japan in the 1880s to the technical aid programmes of the communist countries today, in which training is imparted according to disciplines, sub-disciplines and even specific skills, which are needed in relation to committed programmes.

## Maintaining Communication

Systematic opinion surveys as well as observation and experience suggest that when specialists from developing countries are abroad they lose contact with domestic professional, social, economic and political developments to a high degree. For example, a pilot survey carried out in 1967 in the Greater Boston area in the United States revealed that 90 per cent of Indian students of natural science and technology never saw the bulletins of leading research organizations such as the Council of Scientific and Industrial Research, the Department of Atomic Energy or the Univer-



sity Grants Commission. Foreign student advisers in American universities have indicated that a special government publication, which details opportunities in research and education in India and which is sent to all major American and European universities, is seldom seen by Indian students on their campuses. Even weekly editions of Indian newspapers are seen infrequently.

And so it is perhaps not surprising that India's overseas professionals are unaware of major initiatives being taken by the Government of India to create more opportunities for skilled professionals in, for example, the electronics and aeronautics industries, small scale manufacturing and agricultural research and development. They are continuously exposed to their social, economic and professional developments in their country of study and simultaneously to partial and often slanted reports on India by the mass media of their host country. This converts the information gap into one of cynicism and despondency about the possibility of India's progress in general and, in particular, their own role in it.

To combat this situation, it is imperative that, first, the overseas circulation of the bulletins of research and educational institutions of the developing countries is expanded, with a view to reaching individual students. Second, educational and cultural attachés in the embassies of developing countries in the United States, United Kingdom and Canada should periodically visit universities with large concentrations of their students, not merely to provide information about jobs at home, but to help to solve problems. In this way trust and confidence will be built up. Third, leading scientists and administrators from developing countries should visit major university campuses when they are in North America and Europe. On such occasions the focus should be on scientific and technological programmes at home, trends in scientific, technological and educational policy and so on.

Before they leave their home countries all students and self-sponsored trainees should be required to undertake a "familiarization programme", for which the following model might be considered.

Students who have secured admission to a foreign university could be brought together for discussions and seminars on current and projected scientific and technological prob-

lems and programmes, led by outstanding practitioners from various disciplines. Such meetings would also put the role of the professional in the context of the overall developmental effort of the home country. After a plenary or general session of this kind, during which the seminar leaders would live with the students to ensure informal contact, the students could be broken up into small groups on a disciplinary basis and "teach-ins" could be conducted by specialists. The potential foreign trainees would then be taken round some of the country's major industrial, agricultural, research and educational centres to see the day to day problems of development. The whole programme might then culminate in a meeting with the prime minister or president of the country, at which stage some exhortation may be introduced. Such a scheme might seem far too ambitious and expensive. But India, for example, has been running such a programme, for civil service trainees, for more than ten years. The nature and aims of the effort will be different, but it ought to be found to be at least as effective.

## Recruiting Foreign Professionals

So far the Government of India, for example, has relied almost entirely on the Scientists Pool Programme to enable foreign professionals to be recruited by Indian organizations. There have been indications for some time, however, that this will not suffice. Direct overseas recruitment by employers is necessary. The details of any scheme to undertake such recruitment will vary from country to country, but any such effort should involve at least the preparation of a list indicating all budgeted, but unfilled, vacancies for professionals in laboratories, government departments and universities; there should also be detailed job descriptions in each case, and the maximum emoluments the employer would be prepared to pay to a professional meeting his requirements. A selection panel of professionals, from industry, universities and research institutions, who are not only "senior" enough but whose disciplinary/technological specializations broadly match that of the people to be recruited, should then be sent abroad on a recruiting mission.

Ratio of the Annual Number of Emigrants to the Annual Number of Graduates from the Related Field of Study by Country of Origin or Last Permanent Residence over the Period 1962-1966

|                      | Engineers                |                                     | Natural scientists       |                                     | Physicians               |                                     | Nurses        |                          | Social scientists United States |
|----------------------|--------------------------|-------------------------------------|--------------------------|-------------------------------------|--------------------------|-------------------------------------|---------------|--------------------------|---------------------------------|
|                      | France and United States | France and United States and Canada | France and United States | France and United States and Canada | France and United States | France and United States and Canada | United States | United States and Canada |                                 |
| Europe               |                          |                                     |                          |                                     |                          |                                     |               |                          |                                 |
| Greece               | 23.9                     | 26.6                                | 10.3                     | 12.2                                | 5.9                      | 7.7                                 | 5.1           | 8.3                      | 0.2                             |
| Turkey               | 6.5                      | 6.9                                 | 12.6                     | 15.9                                | 9.8                      | 15.2                                | 1.9           | 2.8                      | 0.1                             |
| Asia                 |                          |                                     |                          |                                     |                          |                                     |               |                          |                                 |
| Burma                | N.a.                     | N.a.                                | N.a.                     | N.a.                                | 1.3                      | 2.9                                 | 0.7           | 1.0                      | N.a.                            |
| Hong Kong            | 264.2                    | 514.2                               | 31.6                     | 59.3                                | 22.2                     | 65.1                                | N.a.          | N.a.                     | 12.3                            |
| India                | 4.4                      | 6.1                                 | N.a.                     | N.a.                                | 0.6                      | 1.6                                 | 0.1           | 1.1                      | 0.1                             |
| Indonesia            | N.a.                     | N.a.                                | N.a.                     | N.a.                                | —                        | 0.1                                 | N.a.          | N.a.                     | N.a.                            |
| Iran                 | 47.5                     | 51.9                                | 12.3                     | 14.1                                | 7.7                      | 10.0                                | 3.3           | 3.9                      | ∞                               |
| Iraq                 | 7.5                      | 9.2                                 | N.a.                     | N.a.                                | 4.3                      | 4.9                                 | N.a.          | N.a.                     | 0.1                             |
| Israel               | 12.7                     | 15.7                                | 15.8                     | 18.6                                | 43.8                     | 54.3                                | 9.4           | 11.4                     | N.a.                            |
| Lebanon              | 33.1                     | 35.5                                | 9.4                      | 10.5                                | 15.2                     | 24.9                                | N.a.          |                          | 0.5                             |
| Malaysia             | N.a.                     | N.a.                                | N.a.                     | N.a.                                | N.a.                     | N.a.                                | N.a.          |                          | N.a.                            |
| Pakistan             | 3.2                      | 9.2                                 | 0.2                      | 0.5                                 | 0.5                      | 1.4                                 | N.a.          |                          | 0.01                            |
| Philippines          | 0.4                      | 2.3                                 | 3.6                      | 18.5                                | 12.0                     | 19.3                                | 3.6           | 35.3                     | 0.03                            |
| Republic of Korea    | 4.0                      |                                     | 2.4                      |                                     | 3.1                      |                                     | 1.1           |                          | 1.5                             |
| Republic of Viet-Nam | 76.3                     |                                     | 17.6                     |                                     | 1.0                      |                                     | N.a.          |                          | N.a.                            |
| Syria                | 54.2                     | 56.5                                | 10.9                     | 11.7                                | 6.7                      | 9.3                                 | N.a.          |                          | 0.8                             |
| Thailand             | 1.0                      |                                     | 0.1                      |                                     | 2.9                      |                                     | 2.6           |                          | 0.04                            |

This table is taken from the Official Records of the UN General Assembly twenty-third session 1968. Annexes to Agenda item 47.

Apart from its portfolio of "professionals needed and funded", it is imperative that the panel has the authority to offer appointments and emoluments to suitable interviewees immediately. If a developing country does not have the necessary mature professionals to staff such panels, United Nations agencies may be requested to provide them as consultants.

A major stumbling block in the return of fully trained professionals to many developing countries has been the fact that they have little choice but to join an organization in their own country. This means that they have to conform to the behavioural and intellectual environments of such organizations, the poverty and rigidity of many of which are deep. It therefore seems necessary to design institutional structures in which overseas professionals would have greater control over their own activities. A design and development estate and a cooperative hospital are two examples which might be considered.

The design and development estate would have many similarities with the industrial estates that already exist in several developing countries—an industrial development corporation would provide land and certain facilities and hire out laboratory and engineering space to individual engineers or partnerships. Engineers returning home would also need low interest loans and foreign exchange allocations. They could then use these resources and their skills to take on contract work for government and other agencies.

The cooperative hospital would have some similarities with the development estate, but would clearly require more planning and organization and also financial aid from the government. A group of medical doctors and hospital administrators could be asked to examine how such a project might be tackled operationally.

## Responsibilities Abroad

It is widely accepted in scientific and academic circles in the United States and the United Kingdom—the principal destinations of professional manpower from the developing nations—that the present immigration policies of these countries are discriminatory, being heavily biased in favour of high level manpower. This situation needs urgent redress if there is to be any credibility behind the protestations that these countries are willing to implement all non-punitive measures to reduce and reverse the loss of talent from developing countries. Operational measures are not difficult to devise. For example, in the United States, three initiatives are immediately possible. First, if the United States wishes to limit the total flow of immigrants, the entry of that number of immigrants should be entirely on a first come first served basis. Second, after completing their training or work all holders of "exchange visitor" visas should be required to return to their home country for a certain period before applying to enter the United States as immigrants. Third, the specified period of stay at home should be increased from two to four years. The US government should then indicate its willingness to apply these changed regulations to those students and other professionals whose national governments make a specific request to this effect. The regulations need not be made to apply to all categories of professionals, but only to those such as medical doctors, who are specifically indicated by the developing country concerned.

In case of some high income countries, an inadequate domestic supply of certain types of manpower is directly responsible for the loss of brains from developing countries. For the past seven or eight years, 25 to 30 per cent of all residents and interns in US hospitals have been from the developing countries. Yet, under pressure from such powerful lobbies as the American Medical Association, the intake of local students into US medical colleges and universities continues to be carefully limited. Consequently, a large

unfulfilled demand for medical services has built up and US hospitals are meeting it by consciously depending on a steady inflow of freshly trained doctors from the developing countries.

It has often been suggested that countries like the United States and the United Kingdom should have, at least in a few universities, courses in natural science and engineering oriented towards the problems of developing countries. But the fact that the level of specialization is often as important as the content and orientation of courses seems to have received less attention. This aspect is important, however, because there is evidence that the probability of a developing country "losing" one of its students abroad is related to whether his study programme involves a first, a second or a third degree. My investigations have revealed that for 300 Indian alumni of the "top ten" American universities, during 1956–1966, the probability of immediate return home after taking a first degree was 50 per cent, after taking a second degree it was 41 per cent and after gaining a PhD it was only 18 per cent. The variance in each case was close to 5 per cent. Similar results have also been obtained by other investigators (private communication from D. D. Henry and C. Susskind).

Although many factors must be taken into account in interpreting these figures, the degree of specialization and probability of return do seem to be related, which is hardly surprising in view of the following arguments. First, although the marginal economic advantage to a first degree holder in acquiring a second degree is not very different in a highly industrialized country from that in a developing country, the differential advantage is significant if he acquires a third degree, because of the nature of the two economies. Thus, when a student from a developing country completes his PhD and then assesses his "comparative market value" in his home country and in the country in which he has studied, the differential in favour of the latter is so great that his choice of whether or not to return home is virtually pre-empted. This, however, is not so in the case of a student who has just acquired a master's degree. Second, educationalists agree that, in most industrialized countries, the PhD involves such intense specialization, at such an impressionable stage of the student's intellectual and cultural development, that his inclinations are heavily oriented, not only towards an academic or research career, but towards one in which he will be able to continue to work in the narrow sub-speciality which he chose as his thesis topic<sup>1</sup>. Such a person has alienated himself from his home country without giving it a chance to secure and hold his interest and commitment.

## Education Overseas

It would thus seem necessary for more students to be urged and, if necessary, required by their own governments to terminate their overseas education after the second degree. The difficulty is that in many universities in the highly industrialized countries, master's degree programmes no longer exist. Thus the governments of developing countries can take the necessary administrative steps only if some of the leading universities in each high-income country, and particularly the United States, start one and two year master's degree courses. These, together with the resolve of developing countries to allow, as far as possible, only first degree holders to study abroad, would also prevent too great an acculturation of the student into the ways of his host country, which seems considerably to influence his decision whether or not to return home. This, however, raises the question of whether preventing such acculturation conflicts with the foreign policy dividends which most industrialized countries expect from their educational and cultural exchange programmes.

It is the responsibility of the high income countries to



assist the developing countries to recruit the latter's professionals who are currently abroad. The kind of assistance needed will depend on the countries involved, but a general problem is to locate engineers in industry and, to some extent, medical doctors whether in service or research. The departments of education of the high income countries should be asked to help the embassies of the developing countries in such location. The governments of the high income countries should also allow their aid funds to be used to support official efforts by a developing country to recruit professionals so located. The annual visits of the selection panels I mentioned earlier might be a case in point.

## Joint Initiatives

Although many different kinds of measures can obviously be devised within the framework of existing bilateral programmes between less and more developed countries, I shall take a specific Indian example to demonstrate the feasibility of such measures. In 1963 the University Grants Commission of the Government of India started a Summer Institutes Programme in collaboration with the United States National Science Foundation and the Agency for International Development of the United States. The purpose of the programme was to run eight week courses for school and college teachers of the natural sciences, to familiarize them with new curricula, teaching methods, and experimental approaches. The institutes were organized on a disciplinary basis and manned by faculty members from American universities, whose numbers have grown from 12 to 179 during the past five years<sup>2</sup>. The programme offers an excellent opportunity for Indian professionals currently holding faculty positions in American universities—of which there were 925 when last counted in 1968<sup>3</sup>—to pay a short visit to their home country. Such visits would enable at least the potential brain drain to renew family ties, visit Indian research, educational and industrial institutions, interact with professional colleagues there, survey employment opportunities and generally become aware at first hand of the challenges (and hazards) of returning for good. Although this initiative is basically joint in nature, the first move must be taken by the United States Government, if for no other reason than that American aid funds are being used to cover a significant part of the cost of this programme.

Student counsellors are needed for postgraduate students from developing countries who are studying abroad. Something must be done to orientate the postgraduate student from a developing country, ideally throughout his programme but at least when he begins to think about the topic for his thesis. Any such scheme requires considerable cooperation on the part of the foreign university, and so a start should probably be made with one country, the United States. The mechanics of the scheme, again using India as an illustration, might be as follows. About 100 students would be selected from those admitted to postgraduate natural science and engineering programmes for the coming academic year, on the basis of their academic quality and, to some extent, the broad relation between the disciplines in which they intend to work and national needs. About twenty counsellors in the same disciplines would be appointed from university faculty, staff of research laboratories and professionals in industry. Each would be "assigned" five students, for whom he would act as "home country interface" on both professional and social matters. Students would have several meetings with their counsellors even before leaving home. (This might be usefully combined with the familiarization programme discussed earlier.)

Soon after the student had gone to the United States, the counsellor would contact the chairman of the university department where his particular students had been registered to discuss the student's background and interests,

courses, research programmes and so on. He would also keep in close touch with his students and do his best to influence their choice of course consistent with the department's programme. During the course the counsellor would provide professional advice and keep him informed of problems at home which illustrate the principles and techniques that the student is learning. As the student approached the time to begin his thesis the counsellor would discuss with the American university the range of research topics available, and their relation to the aptitude of his student and to Indian needs. Eventually several topics would be chosen by the Indian counsellor and the American supervisor for each student to select from. The student would, of course, be kept in the picture.

Such a scheme has two essential merits. First, it gives the student a personal and institutional link with his home country. Second, it provides continuing evidence of its stake in the direction his studies take. But it would not be easy to implement such a scheme.

International corporations have an important part to play in assisting developing country professionals to return home. Foreign governments and commercial companies have to recognize the need for the middle and top management of foreign firms operating in developing countries to be recruited from local nationals. It should not be difficult therefore to encourage foreign governments to urge that companies operating in developing countries recruit local professionals who are working in or have just completed studies in the foreign country concerned.

The problem of the migration of talent from less to more developed countries has attracted considerable attention. There have been many studies (both official and non-official), seminars, conferences and much has been published.

## Dividends Ahead

It is gratifying therefore that all this energy and effort should at last have begun to show dividends. The statistical and conceptual uncertainties of the problem have been so narrowed that an authoritative report can present as its central finding the fact that: "... highly trained personnel from many developing countries are emigrating to a few major developed countries, that the size of this flow is large and that it is increasing at a rapid rate"<sup>4</sup>. It has therefore become less and less possible for individuals, institutions or governments to postpone the day when they will have to face up to this problem, on the excuse that the nature and extent of the migration and its consequences for the countries of emigration are not "adequately" known. There has also been increasing realization that this problem has implications for the developing countries which are just as serious as food shortages or overpopulation. In the past few years therefore several remedial measures of a non-discriminatory and non-coercive nature have been identified and formulated. And yet there is no action. Perhaps the developing countries have not committed "adequate" resources to the measures which are primarily their responsibility. But the fact that the rich nations have shown little recognition of their responsibilities cannot be denied either. Such a state of affairs is perhaps the best indication of the need for international action.

<sup>1</sup> Dandekar, V. M., in *The Brain Drain* (edit. by Adams, W.) (Macmillan, New York, 1968).

<sup>2</sup> *Annual Reports for 1965, 1966, 1967, 1968 and 1969* (University Grants Commission, New Delhi, 1966, 1967, 1968, 1969 and 1970).

<sup>3</sup> *Indians Holding Faculty Positions in USA, Technical Manpower*, Bulletin of the Division of Scientific and Technical Personnel, 10, No. 7 (CSIR, 1968).

<sup>4</sup> *Outflow of Trained Personnel from Developing Countries*, Report of the Secretary-General to the Twenty-Third Session of the UN General Assembly, November 1968, 55 (United Nations, 1968).

# Water in Biological Systems

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**The object of this article is to draw attention to some of the major areas of investigation of the state of water in biochemical and biological systems. Deductions about the role of water in such systems are somewhat speculative, but the situation might be improved.**

THERE is considerable evidence that water does not just function as an inert solvent for biochemical reactions but plays a major role in the mechanistics of biological processes. Perhaps the best known example of this is the concept of hydrophobic bonding, as applied to the explanation of native biopolymer conformations; this was first invoked<sup>1,2</sup> to account for the fact that the non-polar amino-acids, for example in myoglobin<sup>3</sup> and haemoglobin<sup>4</sup>, are resident in the interior of the protein molecule, out of contact with the aqueous environment.

It has been known for some time that the introduction of non-polar moieties into water is accompanied by unfavourable entropy changes which may originate from changes in the hydrogen bonding and/or the arrangement of water molecules in the vicinity of the solute molecules<sup>5</sup>. This phenomenon, hydrophobic hydration, has been studied by various physical techniques, and all the results indicate that both the diffusional and intramolecular modes of water are markedly affected<sup>6</sup>. As a first approximation the formation of a hydrophobic bond between non-polar residues can be regarded as a partial reversal of this process and is thus thermodynamically favoured.

As Scheraga pointed out<sup>7</sup>, the primary contribution to the strength of this hydrophobic bond derives from changes in the "structure" of water when the non-polar groups interact with one another. It is this fact which ensures that, in spite of the high degree of chemical diversity among the amino-acid sequences of proteins such as haemoglobin and myoglobin, their three dimensional (tertiary) structures are so similar.

Before proceeding any further, the meaning of the term "structure", as applied to liquid water, must be clarified. While it is obvious that long lived structures do not exist in a liquid, nevertheless, the physical properties of water indicate a certain degree of intermolecular order, reminiscent of that found in ice. On a suitable time scale, that is  $10^{-11}$  s, ordered arrangements of water molecules, or structures, may well exist, and their lifetimes and vibrational and rotational modes are very sensitive to changes in pressure and temperature and to the presence of solutes. Quantum mechanical calculations suggest<sup>8</sup> that H-bonding of water molecules is a cooperative process, and it is probably this cooperativity, rather than the extensive character of any structural units, which is responsible for this sensitivity to environmental changes. One important consequence of this is that long range interactions are possible between hydrophobic solute molecules, and they can be detected in dilute solutions<sup>9</sup>.

Another striking demonstration of the important role of

water in biological systems is that the substitution of D<sub>2</sub>O for H<sub>2</sub>O in the growth medium leads to alterations of almost every biological trait of a cell<sup>10</sup> and even to complete inhibition of seed germination in some cases<sup>11</sup>. The kinetics of reactions which depend on acid-base catalysis by water, for example, some enzyme reactions<sup>12</sup>, may thus be retarded to such an extent that the overall metabolic balance is severely disturbed. A decrease by a factor of 2–3 in the rate constant of proton transfer reactions is to be expected<sup>13</sup> for such isotope effects. The effect on the subunit aggregation in allosteric proteins, however, can be much greater than the influence on the rate of the enzyme catalysed reaction<sup>14</sup>.

There has developed recently an increasing interest in the state of water in biological systems. A good deal of attention has been paid to water in muscle and brain<sup>15,16</sup> and some results have also appeared for water in hair<sup>18</sup>, in bacterial spores<sup>19</sup> and in plant tissues<sup>20</sup>. The use of oriented fibres has permitted detailed investigations to be made of the hydration of collagen<sup>21,22</sup> and DNA<sup>23</sup>. All these investigations have been based on nuclear magnetic resonance (NMR) spectroscopy. This approach is invaluable in studies of the aqueous component of a complex system, because the water proton NMR signal is so much narrower than the proton signals from the biopolymers and therefore easily distinguished from them. X-ray analysis, on the other hand, is of little use with these systems because of the relatively high mobility of the water molecules.

## What is Not Known

Polar hydrophilic solutes can interact with water to form hydrogen bonds. For example, the hydration of polysaccharides in solution, as deduced from NMR spectra of the aqueous D<sub>2</sub>O component, can be correlated<sup>24</sup> simply with the number of OH groups in the repeating unit of the polymer. In the gel state, however, where there is a more rigid polysaccharide structure, probably containing double helical segments<sup>25</sup>, some overlap of hydration shells seems to occur with repeating units which are sufficiently hydrated. Similarly, for polypeptides, such as polylysine and polyglutamic acid which possess H-bonding groups in the side chain, there is a noticeable effect<sup>26</sup> due to the interaction of water with the polymer, whereas, at equivalent concentrations, lysine and glutamic acid in solution show no observable effects. Moreover, the lifetime of the hydration structure in polyglutamic acid and polyadenylic acid is about  $10^{-4}$  s, for it is only destroyed when the fluctuations in the helix-coil interconversion occur at the maximum rate, that is, at the mid point of the helix-coil transition. Evidence that the interaction between water and biopolymers is long lived supports the view that such interactions are a mediating property with respect to biopolymer conformations in solution. A recent application of this NMR approach, however, makes it clear that the interpretation of experimental data may not be as unambiguous as formerly supposed<sup>27</sup>. It has been suggested that the NMR effects observed from the deuteron relaxation studies which we have mentioned may just be a measure of the number of exchangeable protons on the polymer.



Unlike the hydrophobic, or apolar, hydration phenomenon which has already been mentioned, hydrophilic interactions would be expected to be highly orientation dependent. Warner<sup>28</sup> has pointed out that in many organic molecules the spacing of ether, ester, hydroxy, or carbonyl oxygen atoms is 4.8 Å, which is almost identical with the next nearest neighbour oxygen spacing in a hypothetical ice lattice, expanded to 25°. Thus, provided there are remnants of an ice-like structure in liquid water, cooperative hydrogen bonding could take place without strain to organic molecules such as biotin, 1,4-quinone,  $\beta$ -D-glucose, and triglycerides. Similarly, the polypeptide chains of some proteins, for example, insulin, can adopt strain-free conformations in which all the carbonyl oxygen atoms are spaced 4.8 Å apart. These observations have led to speculations regarding the function of water as a possible "structural cement" in connexion with the association of subunits of tobacco mosaic virus (TMV)<sup>28</sup> and lipid-protein interactions in biological membranes<sup>29</sup>. Self diffusion coefficient measurements on a 4% solution of TMV, evaluated for very brief jump times, have established<sup>30</sup> that only 3% of the water should be considered as highly immobilized. This amounts to four to five water molecules per amino-acid residue in TMV, and so it leaves open the possibility that monolayers of ordered water separate the hydrophilic surfaces of the subunits. In the case of TMV, the argument hinges<sup>28</sup> on whether the X-ray data, which seem to be inconsistent with Warner's hexagonal peptide model, are sufficient to invalidate his proposal.

There is some experimental evidence for this particular concept of hydrophilic hydration, in that one or two interesting correlations have been found<sup>28</sup> between the 4.8 Å spacing of oxygen atoms and chemical and biological reactivity or organic compounds. Our own studies of the physical properties of dilute solutions of carbohydrates which also possess the 4.8 Å spacing of oxygens have shown quite clearly that water can discriminate between molecules as similar as  $\alpha$  and  $\beta$ -methyl pyranosides which only differ in the position (axial against equatorial) of one hydroxy group. It is also clear, however, that the water-solute interaction is of a short range nature in this case, unlike that observed for hydrophobic solutes<sup>9</sup>. This does not necessarily rule out its importance in systems such as membranes where the hydrophilic surfaces may be close together<sup>26</sup>, but much remains to be done to substantiate this hydrophilic hydration hypothesis. Investigations of its general applicability are needed, with particular emphasis on macromolecular surfaces<sup>31</sup>.

New structures are another unknown quantity. It is generally assumed that the structure of water in a region of hydrophilic hydration resembles that of ice I (hexagonal ice), and it has been suggested that, in the vicinity of hydrophobic groups, water adopts a clathrate-type structure<sup>32</sup> such as exists in crystalline gas hydrates<sup>33</sup>. Other structures have, however, also been proposed for water in contact with hydrophilic surfaces. In hydrated collagen, it has been suggested<sup>34,35</sup> that water molecules are arranged in a pentagonal cage network; and the much publicized polywater, prepared by the condensation of water vapour in quartz capillaries<sup>36</sup>, has recently been assigned<sup>37</sup>, although on scant experimental evidence, a polymer structure in which most oxygen atoms are bonded to only three hydrogen atoms. In other cases, such as water in muscle, the aqueous component probably exists in at least two varieties of differing molecular mobility<sup>15,17</sup> which, in turn, are each thought to contain two or more sub-fractions<sup>15,17</sup>. There is, however, insufficient evidence even to begin to speculate on possible structures.

## Role of Water

Much remains to be learnt about the role of water. As far as the structure and thermodynamics of macromolecules are concerned, NMR studies have shown that in certain systems the relaxation rate of water is raised, that is, the

water becomes more solid-like in its motions. Certain predictions can therefore be made about the properties of systems containing such dynamically modified water.

For example, the lipoprotein component of membranes would be expected to induce such modifications in water motions. Whatever the modified molecular configuration may be, the transport properties of any ions present will undoubtedly vary from their behaviour in bulk water. A comparison of ionic transport properties in water and ice<sup>38</sup> shows that any influence which promotes water "structure" would, on the one hand, reduce the mobility of alkali metal ions, and, on the other, enhance the mobility of protons and hydroxyl ions. The slowing down of proton exchange in aqueous solutions containing ions which break up water structure has been established<sup>39</sup>. The relevance of such phenomena to processes involving ionic transport across membranes has been discussed by Klotz<sup>40</sup>.

Having introduced a generalization to the effect that proteins influence water structure, it is necessary to underline the assumptions implicit in such a statement. In what circumstances can proteins be said to have a specific interaction with water? This is an important consideration, because it may relate to the effectiveness of the reciprocal interaction between water and protein, and so to the role of water in controlling protein conformation. The point must be made that anhydrous proteins do not exist, and that the very term protein refers to a polypeptide+water system. The same is true for other biopolymers, and it is known that native conformations can only be maintained in the presence of a critical amount of water which varies with the polymer species.

Of the fibrous proteins investigated so far, from the point of view of hydration structure, collagen has received the most attention. The water is thought to form a pentagonal structure which is bonded to the triple helix. However, the latest view<sup>41</sup> of this system is that the three dimensional structure of the water may not be determined by its interaction with the protein, and the suggestion is made that, whenever water is restricted in a channel of molecular dimensions, as it is in this case, it adopts a particular structure which is not present in the bulk liquid. It is certainly established that when water is constrained by microscopic glass capillaries, its vapour pressure is considerably lower than would be expected on geometric grounds, and consequently its liquid structure is more highly organized<sup>42</sup>. This suggestion of the presence of capillary water in fibrous proteins emphasizes the need for more definitive experiments to test the relevance of structured water in biological systems.

One useful approach which has been taken<sup>43</sup> in the case of solutions of macromolecules involves thermodynamic investigations. Thus, DNA, egg albumen and chymotrypsinogen were seen to exhibit a common thermal denaturation behaviour, indicating that changes in water structure provided the common factor. A similar idea has also been advanced<sup>44,45</sup> to explain why the effect of aqueous anions on the stability of the native structures of biopolymers follows a common sequence, the Hofmeister, or lyotropic, series, for both proteins and nucleic acids. More work along these lines is needed.

At this point it is as well to draw attention to the two schools of thought on the means by which water exercises its thermodynamic control of the conformation of macromolecules. Klotz<sup>1</sup> has argued in favour of apolar hydrate formation, which involves the formation of water cages about hydrophobic groups and is accompanied by a favourable enthalpy change. On the other hand, Kauzmann<sup>2</sup> and Scheraga<sup>7</sup> favour the view that the apolar bands exist between adjacent hydrophobic groups in the biopolymer, and, as mentioned earlier, such a process will be entropy controlled. In the case of myoglobin and haemoglobin, at least in the crystalline state<sup>3,4</sup>, the number of exposed hydrophobic residues has been shown by X-ray analysis to be very small,

which supports the latter hypothesis. In lysozyme<sup>46</sup>, however, and particularly in subtilisin<sup>47</sup>, numerous hydrophobic side chains are situated on the molecular surface, so that a decision between the two theories is not clear cut. In solution systems, it remains to be seen which idea is the more credible.

These problems are not trivial. Since the phenomenon of tertiary and quaternary structure in proteins, and thus of enzyme activity, seems<sup>2,48,49</sup> to be related to interactions of water with non-polar amino-acid side chains, further investigations are clearly indicated.

There is a fair amount of evidence<sup>50</sup> to suggest that general acid-base catalysis is more important for difficult reactions than for processes in which the reactants have marked affinity for each other. This observation may be relevant to enzyme-catalysed reactions, since most biological substrates are non-activated. In fact, since it seems unlikely that any single catalysed reaction step is sufficient to account for the high efficiency of such reactions, the concept of concerted general acid-base catalysis is even more appealing<sup>51</sup>. Thus one or more molecules of water may serve the function of either general acid or general base catalyst<sup>52</sup> and would not appear in the rate law. Indeed, it has been suggested that in the mutarotation of sugars, water acts simultaneously as acid and base catalyst. Such speculations merit investigation in the context of enzyme catalysis.

## Control of Cell Processes

It has been argued<sup>53</sup> that the entire water in the living cell exists in "polarized multilayers", and freezing experiments provide evidence for this<sup>54</sup>. They indicate that, within a muscle cell, ice crystals only grow in the direction of the fibre and, if the fibre is previously twisted, then this will be reflected in the ice spikes<sup>55</sup>. The results of a thermodynamic study suggest<sup>53</sup> that intracellular sodium ions are bonded into this multilayer water structure and that their concentration is lower than in the external medium because of the unfavourable entropy of ionic transfer to the intracellular environment. NMR studies<sup>56,57</sup> of the intracellular sodium ion of muscle have confirmed that the molecular motion of 70% of the sodium ions is restricted to such an extent that it is said to be in a complexed state. Some of the criticisms which were subsequently levelled at these NMR experiments have recently been re-evaluated by independent workers and the original findings confirmed<sup>58</sup>.

The implications of such experiments are that the asymmetric distribution of solutes between intra and extracellular environments might be accounted for without recourse to the concept of specific solute pumps, which so far have been a generally accepted ingredient of cellular behaviour<sup>59</sup>. The state of intracellular water seems crucial to these arguments. It has been found<sup>60</sup> that the diffusion coefficients of various solutes in an intracellular environment are a factor of two lower than in aqueous solution, but the fact that the diffusivities of some cations, anions and non-electrolytes are reduced to the same extent is in direct conflict with the concept<sup>56</sup> of selective ion binding by the macromolecular cell components.

There is need for an interdisciplinary approach. It is always tempting to suggest that one obstacle to further progress is that physiologists and biochemists normally do not consider the behaviour of the aqueous component of complex biological systems and, therefore, miss what may be the common factor in the control of a number of biological processes. On the other hand, it is because physicists and physical chemists have, in general, turned a blind eye to biological systems that concepts such as solute pumps have not been subjected to many critical investigations by sophisticated modern techniques, even though it has been claimed that the total energy needed to operate all the pumps essential to maintain existing intracellular solute concentrations far exceeds the metabolic energy available to the cell<sup>10,53</sup>. An

alternative to the osmotic theory of cell swelling has also been proposed<sup>61</sup>, which relies on the concept of hydration of cell proteins, in an attempt to provide an explanation of the changes in cellular volume which occur when the membrane is permeable to external solutes as well as to water. The development and impetus of theories such as these rely on the physical scientists directing some of their effort to these problems in order to evaluate the extent to which these are valid alternatives or indeed useful supplements to the traditional views on cellular behaviour<sup>62</sup>.

A surprising number of the publications referred to emanate, at least in part, from medical schools in the United States. This illustrates the strong biochemical and biological support from which this type of work benefits. In the biophysics departments in the United Kingdom, the major research effort is concentrated on X-ray diffraction studies, which have led to exciting discoveries of biopolymer structures, but which are not well suited to a study of dynamic behaviour or to a characterization of the aqueous component of the system. Certainly the preliminary work on the state of water could most profitably be carried out by biophysicists or biophysical chemists. Since biologists do not seem to relish being converted to biophysicists<sup>63</sup>, it is unlikely that, left to themselves, they would initiate such work.

The efforts of physical chemists have been directed almost exclusively towards studies of model systems. One must, however, always beware of too hasty a choice of system. In this sense, aqueous solutions of synthetic polypeptides have been shown not to serve as good thermodynamic models for globular proteins<sup>64</sup>. In some cases the systems selected for investigation have been unusual, to say the least. A study of the effects of modifying the water-gluten interactions in doughs<sup>65</sup> has shown promise in relation to the action of anaesthetics, depressants and stimulants on the nervous system. A start has also been made on what might be called real systems. The function of water in controlling membrane permeability<sup>66,67</sup> and narcosis<sup>68,69</sup> is receiving attention. An elegant investigation has been reported<sup>70</sup> of the state of intracellular water and the change in membrane permeability resulting from nerve action. It is in areas such as these, including studies of the chemistry of those protein reactions which may reflect the role of water<sup>71</sup>, that the liaison between biochemists and biophysicists is invaluable.

## Other Problems

Practical problems also include the state of water in biological systems. The techniques most useful here are those which can, in principle, focus on one component of a multi-component system. The methods of NMR, dielectric and possibly ultrasonic relaxation fall into this category. At the same time, of course, it is useful in biopolymer systems to look for corresponding changes in the state of the polymer. Very high resolution NMR spectroscopy (220 MHz) is finding some applications here<sup>72</sup>, an interesting example being that it can detect some difference in the conformation of the cyano complex of myoglobin in H<sub>2</sub>O and D<sub>2</sub>O solutions (reported by B. Sheard at the international conference on magnetic resonance in biological systems, Oxford, 1970). The recent introduction of time domain techniques to study dielectric relaxation<sup>73,74</sup> should ensure that investigations of the simultaneous reorganization of both the aqueous and other components of solutions become more widespread in the future.

The role of water poses other practical problems. Results of relaxation studies are often discussed<sup>75,76</sup> in terms of the short range interaction between water and a solute, as specific hydration, or bound water. It has been suggested that this approach is not as useful to the elucidation of the role of water in solutions as is a measure of the overall change in water structure accompanying a transformation of the system. Thus, such diverse processes as the transfer of



electrolytes or non-electrolytes from  $H_2O^7$  to other solvent media, the binding of negatively charged inhibitors to  $\alpha$ -chymotrypsin<sup>78</sup> and the effect of ethanol on transition I of ribonuclease A<sup>79</sup> exhibit what is known as compensation behaviour. The enthalpy change,  $\Delta H$ , is linearly related to the entropy change,  $\Delta S$ , with a proportionality constant or "compensation temperature" in the range 250–320 K. This property of compensation is believed to be unique to aqueous solution process<sup>80</sup>. It cannot originate from specific hydration of the solutes. It seems to be related to a property of water which is sensitive to long range interactions. Thermodynamic measurements, depending as they do on time-averaged interactions, seem to offer a more sensitive probe of the modulating influence of water on solute properties than do the spectroscopic methods.

Any proposal model must be consistent with the thermodynamics of the system. To make a quantitative estimate of the contribution played by the aqueous component, the thermodynamics of all the processes occurring simultaneously must first be evaluated. This rules out *in vivo* studies at the moment, because of a lack of the detailed knowledge required to make sense of them<sup>81</sup>. Thus the major thermodynamic effort must first be focused on biochemical rather than biological systems.

Commercial calorimeters are available with adequate sensitivity, and the calorimetric determination of equilibrium constants<sup>82</sup> offers the possibility of determining free energies, in addition to enthalpies and heat capacities, without incurring further cost of additional equipment.

We have presented some evidence that the motions of water molecules in biological systems differ from those in bulk water. It remains to be seen to what extent the structure of water is also modified. It is not anticipated that much progress can be expected in this area, while the intermolecular nature of bulk water itself is still a matter of lively controversy<sup>83</sup>.

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# Doubts about Mach's Principle

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**Mach's principle as frequently described may be illusory, but attempts at deeper formulations are difficult to interpret.**

THE purpose of this article is to suggest that Mach's principle in any simple form is largely illusory, although it is difficult to give it a meaning in any more sophisticated form. The possible novelty of the argument presented in the first part is that it gives a simple physical reason for the apparent connexion between local inertial properties and remote matter in the universe.

Laboratory experiments with Foucault pendulums, gyroscopes or other entirely local means enable us to establish a unique local frame of reference that we say is not rotating. We then ask with respect to what it is non-rotating. A standard of non-rotation is obviously not provided by, say, the Earth, the Sun or the Galaxy because we can speak meaningfully of the non-zero rotation of each of these systems; there remains only the background of the remotest objects in the universe.

## Fixed Directions

The key to our discussion is that, if we look at a very remote object, we look in a fixed direction. If we are at O and A, B are any two adjacent objects in the sky, then because the relative speed of mutual separation or approach of A and B cannot exceed the speed of light  $c$ , the rate of change of the angle AOB becomes as small as we please simply by taking the distances OA, OB to be sufficiently great. Therefore, so far as directions are concerned, we obtain a rigid frame of reference simply by looking at very distant objects. If we take, say, a potato and insert knitting needles pointing at any number of the remotest galaxies, A, B, C, . . . , then they can be kept pointing at A, B, C, . . . for ever without any readjustment. This is really the old concept of the "fixed stars"; it is also the concept used in stabilizing a space-vehicle by keeping it "locked" onto two or more stars. If we suppose we are in the world of classical physics, the concept works well in practice; in principle, however, classical theory does not require the property to hold good because it sets no bound to the relative speed of objects like A and B. The property depends upon the finiteness of  $c$  and the requirement that the relative speed of any two neighbouring material objects is less than  $c$ , as demanded by special relativity theory<sup>1</sup>.

The property under consideration is in fact most clearly presented in terms of the space-time of special relativity. We recall that in this space-time the aggregate of null-cones is an absolute feature; it is "there" to be explored by any observer but it is independent of any observer. Let  $l$  be the world-line of the observer O and let  $a, b, c, \dots$  be the world-lines of galaxies, A, B, C, . . . . If  $\gamma$  is a generator of the backward null-cone of some event E in  $l$ , then through any other event  $\bar{E}$  in  $l$  there is a unique null-line  $\bar{\gamma}$ , parallel to  $\gamma$ . Suppose the world-line  $a$  happens to meet  $\gamma$  so that  $\gamma$  is the world-line of a photon

by which O, at the event E, sees A. Then through  $\bar{E}$  there is a generator  $\gamma^*$  of the backward null-cone of  $\bar{E}$  that meets  $a$ ; this  $\gamma^*$  is the world-line of the photon by which O, at the event  $\bar{E}$ , sees A. The property we have been discussing is that, if A is taken to be farther and farther away from O, then in the limit  $\gamma^* \equiv \bar{\gamma}$ . We may say simply that by watching any very remote object we pick out a succession of parallel null-lines through the succession of events on our own world-line. There is, moreover, no other purely optical way of picking out parallel null world-lines. But the remote object is merely a means to an end, which is to explore the null-cone structure in the vicinity of our own world-line. We may note that short portions near our world-line of the succession of parallel null-lines form a two-dimensional ribbon in the four-space which is also the history of one of the knitting needles in the illustration.

The natural inference from Newton's rotating pail of water, Foucault's pendulum and so on is that a mechanical system also explores or detects the local null-cone structure. Then, naturally, we obtain the same measure of any angular velocity from the mechanical system as we do by looking at very distant objects simply because looking at very distant objects happens to be a convenient alternative way of exploring the same local null-cone structure. There is no justification for saying that the remote material in the universe influences or determines local inertial properties any more than, for instance, a distant church steeple influences a telescope if we view it in order to collimate the telescope.

## General Relativity

The argument so far is expressed in terms of special relativity but it is equally valid in general relativity (with a certain elaboration mentioned below). In fact, the conclusion becomes even more natural, for we can make a comparison with the case of gravitation. If we drop an apple, it falls towards the centre of the Earth, but how does it know where the centre of the Earth is? This, essentially, is the problem about which Newton expressly declined to speculate. But Einstein gave the simple answer that the apple knows nothing about the Earth or its centre—it merely explores or detects the local curvature of space-time. According to general relativity, the Earth itself is one feature of space-time in our vicinity, and the Earth's gravitational field is another feature of the same space-time. These two features are equally real in physics as expressed by general relativity. All we have been saying is that the null-geodesic structure in the space-time is also equally real and Newton's apple or pail of water can detect this feature also. The way in which a test-body detects these properties is not understood at present, but we are asking far less for the body to detect something at its own position than for it to determine its behaviour with reference to galaxies perhaps  $10^{10}$  light years distant.

In the space-time of general relativity, the null-geodesic structure is that of the tangent flat space-time to a first approximation. Local features are therefore just the same as in special relativity and, in particular, local inertial properties do not depend on the curvature of space-time elsewhere and so they do not depend on the distribution of matter in the universe. Also the observer may still select null-geodesics by



looking at distant objects; for this purpose it does not matter how much the geodesics are curved. The curvature should, however, not vary significantly with the observer's time. For instance, if a nearby massive body moves across the background of remote galaxies, it will produce the "gravitational lens" effect. This has no influence whatever on local inertial properties and affects only the possibility of using a remote galaxy that happens to be almost in line with the moving body in order to get a "fixed" direction. This illustrates the elaboration to which I have referred. A more realistic, but more complicated, example of the same type of effect was treated by Eddington<sup>2</sup>.

## Mach's Principle

If the discussion is correct, it shows the so-called Mach principle—in the way it seems to be usually understood—to be largely illusory. The principle seems never to have been given a simple formulation much beyond a vague statement that inertial properties are determined by the actual contents of the universe in the large. Apart from its vagueness, any such assertion is unsatisfactory because it could not be tested by experiment; we cannot rearrange the contents of the universe in the large to find whether inertial properties are thereby changed. The most we can do is to see whether inertial properties are related to any irregularities in the actual distribution of matter. No such effect has ever been detected, which agrees entirely with the views expressed here.

It is sometimes said that special relativity ought not to account for inertia because, from the standpoint of general relativity, it is the case of empty space-time. But from this standpoint, special relativity is the case of a particular field, not the case of no field at all. According to the view expressed here, the particular case is indeed that in which only inertial properties, and not gravitational properties, are represented. It is, furthermore, natural to find a connexion between inertial properties and null-geodesics. A null-geodesic is the world-line of a photon and a photon has zero rest-mass, so we may say that a null-geodesic is the world-line of a particle whose mass is wholly inertial. These conclusions are generally supported by the experimental work of Sagnac and others (see ref. 3). I find also that the conclusions agree closely with those repeatedly stated by Eddington<sup>2,4</sup>.

## Deeper Formulation

The subject may certainly be considered at a deeper level. This is well illustrated by a paper of Lynden-Bell<sup>5</sup>. Indeed, I had drafted the foregoing arguments at about the time that this paper appeared and, in view of Lynden-Bell's discussion, mine appeared not to get very far. He proposed a programme of investigation which has apparently never been carried out nor, indeed, shown to be possible. It therefore seems worthwhile placing my rather elementary arguments on record, together with some remarks prompted by further reflexion on Lynden-Bell's work.

All such discussions are made difficult by the simple fact that we can properly discuss a theory only in terms of itself. We might, for example, try to ask in some quite general sense whether space-time can exist without matter. But if we try to say what we mean by matter and by space-time we must employ a precisely formulated theory. Then what we are really asking, it seems, is whether in the theory it is sensible to say that what the theory calls matter generates what the theory calls space-time. We cannot expect to give a once-and-for-all answer as regards the actual world of experience; we can only say whether a theory that gives one sort of answer corresponds to experience better than a theory that gives another sort.

Lynden-Bell seems to follow this interpretation: he does discuss a particular theory, the theory of general relativity. As I understand his approach, he asks whether some theoretical models of the physical world are such that we can assert

that the matter in them causes, or generates, the space-time. In his case, of course, the meaning of the terms, the models, and the inferences about them, have all to be provided strictly by the theory of general relativity itself. If such models can be found, Lynden-Bell would call them Machian or Berkeleyan. As the outcome of some penetrating analysis, he formulates a programme to determine which models are Machian in this sense. Unfortunately the programme would be difficult to carry out, and Lynden-Bell admits that his Mach condition may be "so restrictive that no universe satisfies it".

Lynden-Bell's thoughts about general relativity go very deep, but in a sense general relativity is not a deep theory—at any rate not by design, though maybe it is by accident. It starts with Riemannian geometry (with a certain signature), and proposes to use this as a model of some aspects of the physical world, when a certain derived tensor (the Einstein tensor) is interpreted as the matter tensor (with a particular conversion factor for practical convenience) and certain other features are interpreted as representing the spatial, temporal, inertial, gravitational relationships of the matter so represented. The theory is essentially tentative; it asks whether any of the connexions between these factors imposed by the geometry may be of physical interest. Obviously the theory cannot tell us what sorts of matter do or can exist, and no actual matter is bound to behave in accordance with the theory. The theory is nevertheless recognized to be an "interesting" one, and there can be no going back on some of the lessons learnt from it.

The entity which we here call geometry is one single entity that is a model of a considerable amount of physics. One feature of the geometry gives the result of the operations that an observer in the model would call measuring the density of matter; another feature gives what an observer would find when he says he is observing or measuring the inertial properties of matter, and so on. But within the strict bounds of usage of the theory it is not sensible to say that there is a region of the model in which no matter is present because the theory does not distinguish between space-time and matter. There is the one inclusive entity and it is not even permitted to say that matter is an aspect of this entity, only that the results of certain operations within the model, that we call density of matter, stress and so on, are aspects of the entity.

It is hard to see, with this strict interpretation, what place Mach's ideas can occupy in the theory. Lynden-Bell writes, for example, that "in any truly Machian theory space-time itself must be caused by the matter and in this theory it may be considered as propagating over itself out from the matter". On the present interpretation this seems to be saying that the model must be caused by the model. It is hard to see what this could mean.

It is, of course, possible to formulate approximations in general relativity and in these approximations to draw certain distinctions that disappear again when the approximations are removed. It is possible to assert, in such approximations, that "matter" having zero density and stress does not contribute to the gravitational field but does contribute to the inertial field. For some metaphysical reason, physicists (and Dr Berkeley) may regard this as a defect in the theory. Or they may say that we should restrict consideration to a subset of general relativity models (if such exists) having some feature such as that required by Lynden-Bell.

It does seem to me, however, that the discussion of Mach's principle in the context of general relativity is given some significance only by retaining concepts of pre-general relativity physics. I consider that Mach's principle has never been formulated strictly within the concepts of general relativity.

## The Cosmological Constant

There is, however, a different way of making general relativity possibly more attractive from this general viewpoint. This is to assert that the general relativity models determine

the properties of matter only to within the degree of arbitrariness indicated by the "cosmological terms", in which the cosmological constant is regarded as a genuine arbitrary constant so far as general relativity itself is concerned. We have remarked that the theory cannot be said to be a deep theory by deliberate design; nevertheless, one of its interesting features may be that through the cosmological terms it does leave scope for a more sophisticated determination of the zero-points of density and stress. We might then wish to claim that the cosmological terms express the extent to which matter may be said to determine the inertial features of space-time. That is to say, such an assertion may be given a meaning when the cosmological terms are retained that it would not

have without them. In other words, without sacrificing anything from general relativity, the cosmological terms may, almost literally, give more substance to its ideas. In this way, it may still further weaken the demand for the elusive principle of Mach.

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# A Prototype Live Oral Cholera Vaccine

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**Using the methods of molecular genetics it should be possible to develop a cholera vaccine that produces long lasting immunity. To this end *Vibrio cholerae* mutants deficient in choleraenic activity have been isolated.**

THE cholera vaccines that are now available have been unsatisfactory for controlling the disease in countries where it is endemic. They do not produce adequate long lasting immunity (more than approximately 3–6 months) with one dose<sup>1,2</sup>, and it is impractical to give multiple revaccinations on a massive scale in the affected areas. I describe here procedures for the development of a vaccine with long lasting efficacy.

Cholera victims suffer from a fulminant diarrhoea resulting in severe dehydration and electrolyte imbalance. The diarrhoea is probably caused by a protein factor (toxin) produced by the causative agent, *Vibrio cholerae*<sup>3,4</sup>. This choleraenic toxin can be assayed by its ability to cause in animals a simulated diarrhoea; that is, accumulation of fluid in a ligatured segment of small intestine when the factor is injected into the lumen of the segment. In the canine system the parameters of the fluid and electrolyte alterations are similar to those obtained when live vibrio cells are injected into the intestinal lumen<sup>5,6</sup>. The toxin quickly and apparently irreversibly binds to some substance in the intestinal mucosa. Antibodies directed against the toxin neutralize its activity in the intestinal segment if they are injected before the toxin, but are ineffective if injected 5 min after injection of the toxin<sup>7</sup>. These results parallel the clinical findings in human cholera for which the incubation period can be as short as 1 day<sup>8</sup> and, once the diarrhoea starts, little can be done to shorten substantially its duration or magnitude. (Therapy is basically directed at maintaining fluid and electrolyte balance until the diarrhoea, which is self-limiting, ceases.)

The existing vaccines are preparations of killed vibrio whole cells or cell wall products administered by injection, and

probably confer protection by inducing intestinal antibodies (coproantibodies) that act on the bacteria, presumably with a vibriocidal (bacteriocidal) effect; the degree of immunity does correlate well with serum vibriocidal antibody titre<sup>9</sup>. It is thus not surprising that these vaccines produce immunity for only a few months. Unless the antibody titre is already sufficiently high to kill them very quickly, infecting vibrios would produce toxin that would bind to the intestine, causing an outpouring of fluid before a secondary anamnestic response could produce enough antibodies to have a protective effect. An inactivated choleraenic toxin (toxoid) vaccine, which is now being prepared for field testing (personal communication from J. Seal), might somewhat prolong the period of protection but is unlikely to produce long lasting immunity for the same reasons that apply to the existing vaccines.

A vaccine is required which will continue to act antigenically for a prolonged period. This can be accomplished with a particular kind of oral, live bacterial vaccine. The idea of using a live vaccine for cholera, in itself, is not new. Mukerjee and co-workers have been studying naturally occurring non-pathogenic vibrios for this purpose<sup>10</sup>. It should, however, be possible to improve greatly the efficacy of live cholera vaccines by preparing them from bacteria in which non-pathogenicity has been induced by a very specific genetic mutation. The mutation would be in the structural gene for choleraenic toxin and would result in the production of a protein deficient in all biological activity (with respect to diarrhoea production) but with normal antigenic activity. The mutant strain growing in the intestine could induce antibodies directed against the bacteria and the toxin. Immunization could be effected by ingestion of a lyophilized culture of the mutant bacterial strain protected from gastric secretions by an enteric coated capsule, which would remain intact until it reached the small intestine.

Such a vaccine, however, could not provide long term protection because *V. cholerae* do not survive in the intestine for more than a few days. This could be overcome by transferring the mutated structural gene for toxin from *V. cholerae* to a bacterial strain that is normally part of the intestinal flora. The recombinant strain could then permanently reside in the intestine and continually produce the antigen that induces choleraenic toxin neutralizing antibodies. Such inter-species genetic manipulation has been demonstrated for other gram negative bacillary species and genetic recombination between

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different strains of *V. cholerae* has also been reported<sup>11</sup>. The transfer of genetic information for a somatic antigen from *V. cholerae* to *Proteus vulgaris* via a drug resistance factor has been demonstrated (personal communication from L. M. Prescott).

As with any live vaccine, precaution must be taken to ensure that the vaccine strain does not revert to a pathogenic state. The possibility of reversion by back mutation can be eliminated by making the original mutation a "deletion" mutation; that is, one in which a segment of DNA is removed. Deletion mutations do not revert. They can be induced in bacteria by treatment with nitrous acid<sup>12</sup>. Of course the deletion would have to be short enough to retain the antigenicity of the mutant toxin product. Studies of *Escherichia coli* strains carrying mutations in the  $\beta$ -galactosidase gene indicate that a substantial portion of a protein can be missing without its immunological properties being greatly affected<sup>13</sup>.

*Vibrio* deletion mutants could conceivably revert by recombination; that is, by a crossing-over between the DNA of the mutant part of the *vibrio* toxin gene and some similarly structured portion of a non-homologous gene from another bacterial species that also inhabits the gastrointestinal tract. There is evidence that this occurrence is most unlikely. Reversion has not been observed for other bacterial deletion mutants when extra non-homologous genetic material (e.g., episomes) has been introduced into the mutant cell.

## Genetic Studies

Mutants deficient in choleraemic activity were obtained by a method based on the close association of choleraemic activity with another activity, a vascular permeability activity, which also appears in *vibrio* culture medium filtrates and causes a localized oedema and induration when the filtrate is injected intracutaneously into certain animals<sup>14</sup>. The factor responsible for this second activity has been termed permeability factor (PF). That the association of the two activities continues in varying bacterial growth conditions<sup>15</sup> and separation procedures<sup>3</sup> (only recently has physical separation of the two activities been reported<sup>16</sup>) suggested that some mutants isolated as deficient in PF activity may also have a loss of choleraemic activity. This would occur because either the PF and choleraemic activities, after all, reside on the same protein, or biosynthesis of the two factors is coordinately regulated. In the latter condition, both activities could be eliminated by a single mutation that affects a regulatory gene.

Using this rationale, choleraemic mutants were obtained by nonselectively isolating mutants deficient in PF activity. *V. cholerae* Inaba 569B was mutagenized by three cycles of growth overnight at 37° C in 3% peptone broth containing 60 µg/ml. of N-methyl-N'-nitro-N-nitrosoguanidine (NTG)<sup>17</sup>, a potent mutagen. The culture was diluted fifty-fold into fresh medium containing NTG before each start of the next cycle. Although NTG is not known to induce deletion mutants, it was used in this preliminary study instead of nitrous acid because of its much greater potency. Using this method the fraction of cells resistant to 25 µg/ml. of streptomycin increased from  $<10^{-8}$  to  $10^{-6}$ .

The mutagenized cells were cloned and individual colonies were tested for production of PF activity. To minimize isolating mutants that produced a low level of PF factor only because of poor bacterial growth, colonies with irregular morphology and size were avoided. The colonies were inoculated into 1 ml. of peptone broth and incubated at 37° C in a reciprocal shaker. After a culture became noticeably turbid the culture medium was cleared of living bacteria by centrifuging and shaking with a few drops of chloroform. A sample (0.1 ml.) was injected intracutaneously into a rabbit and 16–24 h later the oedema of each injected area was compared with that for a control area, which had been injected with fresh sterile medium also treated with chloroform. Test areas showing no more oedema than the control were scored as negative. The control area

usually had no oedema. The colonies exhibiting no activity were again cultured and tested as above. A total of 2,000 colonies were examined in this way. This initial screening procedure was crude and insensitive. For any one rabbit, 10–30% of the injected areas were negative on the first test and of these almost as high a percentage was negative on the repeat test. Nevertheless most strains that were negative twice in the initial screening procedure were also negative by the following more sensitive test. Each strain was inoculated into 10 ml. of peptone broth in a 250 ml. Erlenmeyer flask, which was incubated overnight at 30° C in a rotary incubator. The next day, after being cleared of bacteria by centrifugation and 'Millipore' filtration, the culture medium was assayed for PF using the procedure of Craig<sup>14</sup>. In this procedure the permeability effect is more precisely quantified by intravenous injection of a dye 23 h after the injection of PF. The dye attaches to plasma proteins passing into the oedematous area resulting in a coloured spot on the surface of the skin. In the present study Evans blue dye was used. The unit of PF activity is the blueing dose. One blueing dose is the least amount of PF that will result in a blue spot of 5 mm diameter. Strains that produced less than 5% of the wild type PF activity were tested for choleraemic activity. A 2 ml. sample of a culture, which had been incubated overnight at 30° C in a rotary shaker as I have described, was injected into a 10 cm long ligatured segment of rabbit ileum. After 16–18 h the volume of fluid in the lumen of the segment and the weight of the segment after removal of fluid were determined. Of the forty-three mutant strains that eventually proved to be deficient in PF activity, thirty had the same growth rate as wild type as measured turbidimetrically. Of these, eighteen exhibited no choleraemic activity. The data for four of these mutants are given in Table 1.

Since mutants deficient in PF activity could be obtained so easily, an attempt was made to isolate choleraemic toxin mutants directly. The bacteria were grown through three cycles of NTG-containing peptone broth, cloned, and tested for choleraemic activity as we have described. Of the 100 colonies tested, two had detectably decreased choleraemic

**Table 1** Production of PF and Choleraemic Activity

| Strain    | Blueing doses/ml. of culture media filtrate | Fluid accumulation (ml./g of intestine) |
|-----------|---------------------------------------------|-----------------------------------------|
| Wild-type | 1,640                                       | 7.6                                     |
| T30       | <10                                         | <0.1                                    |
| T34       | <10                                         | <0.1                                    |
| T64       | <10                                         | <0.1                                    |
| B56       | <10                                         | <0.1                                    |

**Table 2** Induction of Vibriocidal Antibodies by Mutant Strains

| Rabbit | Injected strain | Serum vibriocidal antibody titre |                 |                 |                 |                 |
|--------|-----------------|----------------------------------|-----------------|-----------------|-----------------|-----------------|
|        |                 | 1st week                         | 2nd week        | 3rd week        | 4th week        | 5th week        |
| 1      | T30             | 10 <sup>1</sup>                  | 10 <sup>1</sup> | 10 <sup>7</sup> | 10 <sup>6</sup> | 10 <sup>7</sup> |
| 2      | T34             | 10 <sup>1</sup>                  | 10 <sup>1</sup> | 10 <sup>5</sup> | 10 <sup>5</sup> | 10 <sup>6</sup> |
| 3      | T64             | 10 <sup>1</sup>                  | 10 <sup>5</sup> | 10 <sup>5</sup> | 10 <sup>7</sup> | 10 <sup>6</sup> |
| 4      | B56             | 10 <sup>1</sup>                  | 10 <sup>6</sup> | 10 <sup>5</sup> | 10 <sup>7</sup> | 10 <sup>6</sup> |

Two parts log phase culture of wild type 569B, one part serum (each appropriately diluted in normal saline) and one part guinea-pig complement diluted 1:20 in normal saline–0.1% peptone solution were incubated for 1 h at 37° C. The final concentration of bacteria was 1,000 to 3,000 cells per ml. After incubation, 0.1 ml. was spread in duplicate on brain heart infusion (Difco) agar plates, incubated overnight at 37° C, and the colonies were counted. The control sample had normal saline substituted for serum. The titres listed are the highest ten-fold serial dilution of serum that permitted a bacterial survival less than 25% of that for the control. Antibody titres listed under the column designated first week are the original preinjection titres.

**Table 3** Induction of Vibriocidal Antibodies by Live and Dead Strains

| Rabbit | Injected strain | Serum vibriocidal antibody titre |                 |                 |                 |
|--------|-----------------|----------------------------------|-----------------|-----------------|-----------------|
|        |                 | 1st week                         | 2nd week        | 3rd week        | 4th week        |
| 5      | T34             | 10 <sup>1</sup>                  | 10 <sup>2</sup> | —               | 10 <sup>4</sup> |
| 6      | T34 (dead)      | 10 <sup>1</sup>                  | 10 <sup>3</sup> | —               | 10 <sup>4</sup> |
| 7      | T34             | 10 <sup>1</sup>                  | 10 <sup>3</sup> | 10 <sup>3</sup> | 10 <sup>3</sup> |
| 8      | T34 (dead)      | 10 <sup>0</sup>                  | 10 <sup>2</sup> | 10 <sup>3</sup> | 10 <sup>3</sup> |

activity. One mutant strain caused accumulation of 0.4 ml. of fluid/g of intestine and has a decreased PF production, both of which could be attributed to poor growth in broth. The other had a normal growth rate in broth, produced a normal level of PF activity and only a slightly reduced level of choleraemic activity (fluid accumulation of 1.4 ml./g of intestine). Neither mutant strain would be useful for a vaccine but this study demonstrates that choleraemic toxin mutants can be isolated directly with some ease.

The attempts to transfer the gene for choleraemic toxin from *V. cholerae* to *E. coli* were unsuccessful. *E. coli* was chosen because it is a member of the intestinal flora, its genetics is the best characterized, and it is sufficiently related to *V. cholerae* to allow drug resistance factors to be transferred between the two species<sup>18</sup>. The techniques of Bhaskaran *et al.*<sup>19</sup> were used in the genetic recombination studies. The plan was to select for recombinants involving genes common to both species (for example, genes for metabolism of amino-acids) and then look for choleraemic activity in the *E. coli* recombinants\*. However, whereas intra-species (*V. cholerae* × *V. cholerae* and *E. coli* × *E. coli*) recombination occurred at a normal frequency, no inter-species recombination was detected within the limits of sensitivity, which was 10<sup>-7</sup> to 10<sup>-9</sup> depending on the strain and genetic marker selection. There was also no detectable transfer of markers from *E. coli* to *V. cholerae* even when high frequency recombination (Hfr) strains of *E. coli* were used as donors. Crosses involving all combinations of donor and recipient strains were attempted, and selection was made for each marker one at a time. Additional experiments along these lines are required.

## Immunological Studies

The four mutant strains described in Table 1 were tested for ability to induce antibodies in conditions simulating vaccination by the oral administration of an enteric coated capsule of lyophilized live bacteria. Overnight broth cultures of bacteria were centrifuged, the bacterial pellet was resuspended in 10% skimmed milk, and 0.5 ml. samples of the bacteria-milk suspensions were lyophilized. Later the lyophilized culture was resuspended in 2 ml. of normal saline, assayed for viable bacteria and injected by means of a 26 gauge needle into the ileum of a rabbit after laparotomy. A blood sample was obtained by cardiac puncture before injection of the bacteria. This procedure was repeated at weekly intervals for a further 3 weeks. At the beginning of the fifth week only a blood sample was taken. Each week between 2 × 10<sup>7</sup> and 2 × 10<sup>8</sup> viable bacteria were injected. The serum for each week was titred for vibriocidal and PF neutralizing activity. As Table 2, shows, each strain induced high titres of vibriocidal antibody within 2 weeks of the initial injection. However, none of the sera demonstrated any ability to neutralize the PF activity of a wild type culture medium

\* Strains used were *V. cholerae* V58P+ and V63P- and *E. coli* AB57F-, AB257Hfr, AB259Hfr, AB311Hfr, AB312Hfr and AB313 Hfr. These strains have mutations in one or more of the genes for synthesis of threonine, leucine, histidine, isoleucine, valine, arginine, proline, methionine and purine, utilization of maltose, and resistance to streptomycin. P and F are the sex factors for *V. cholerae* and *E. coli* respectively.

filtrate even when the serum incubated with the filtrate was diluted as little as ten-fold.

These results suggest that the PF trait was not expressed in the intestine. There is evidence that the bacteria injected into the ileum had a very low survival. Cultures from rectal swabs, obtained from the rabbits every few days after injection of bacteria, failed to grow out vibrio. In another experiment the antigenicity of killed bacteria was compared to that of live bacteria. A lyophilized culture of the mutant strain T34 was resuspended in normal saline, centrifuged, again resuspended in normal saline and divided into two equal portions. One sample was immediately injected into the ileum of a rabbit. The other sample was irradiated with ultraviolet light for 2 h—a dose that killed all the bacteria—and then injected into the ileum of another rabbit. For this experiment, bacteria were injected only during the first week. The results, which are summarized in the first two rows of Table 3, show no difference in the ability of the two cultures to induce vibriocidal antibodies. The experiment was repeated with the live culture suspended in 10 ml. of peptone broth to facilitate multiplication in the gut while the dead culture was suspended in 10 ml. of normal saline. The same results were obtained as described in the last two rows of Table 3.

## Production of a Vaccine

Except for the transfer of the gene for choleraemic toxin to another strain, there are no barriers to the development of the proposed vaccine. The proper mutant can surely be obtained. Even if genetic transfer of the toxin gene cannot be accomplished, the mutant vibrio strain should in itself be able to induce a high level of immunity for a short while. Mukerjee's group has obtained evidence that it is possible to induce toxin-neutralizing antibodies by intra-intestinal immunization with a live bacterial vaccine<sup>20</sup>. The ease of administration should make adequate frequent revaccination practical.

The exact genetic sites of the mutations in the mutants I have discussed have yet to be determined. It is interesting that the PF activity mutants differ with respect to the presence or absence of choleraemic activity. Acrylamide gel patterns of media filtrate extracts from cultures of these mutants also differ. These studies will be the subject of a future publication.

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# Kinetic Evidence for Incorrectly Folded Intermediate States in the Refolding of Denatured Proteins

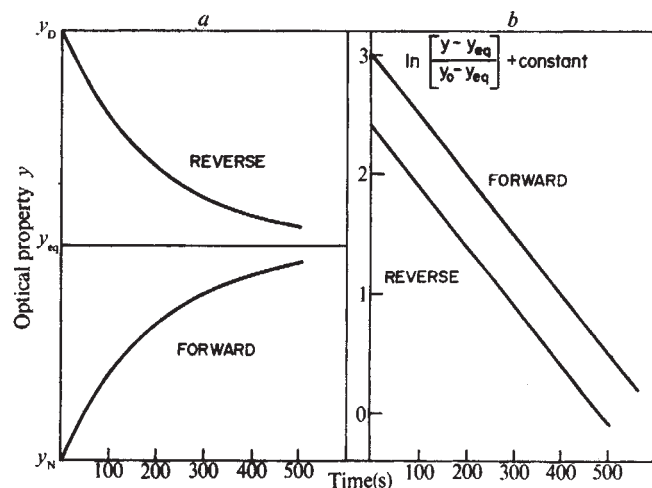
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**Kinetic studies of the denaturation and renaturation of proteins indicate metastable intermediates which are not on the direct pathway between native and denatured states. This suggests that the initial steps in the folding of a structureless polypeptide chain may often be rapidly reversed and without influence on the ultimate result.**

THE reversible transition between native (N) and denatured (D) states of proteins is often a two-state process in the sense that states other than N and D are never present in experimentally significant amounts during the reaction<sup>1</sup>. One manifestation of this is that the change in any optical property  $y$  obeys first order kinetics or

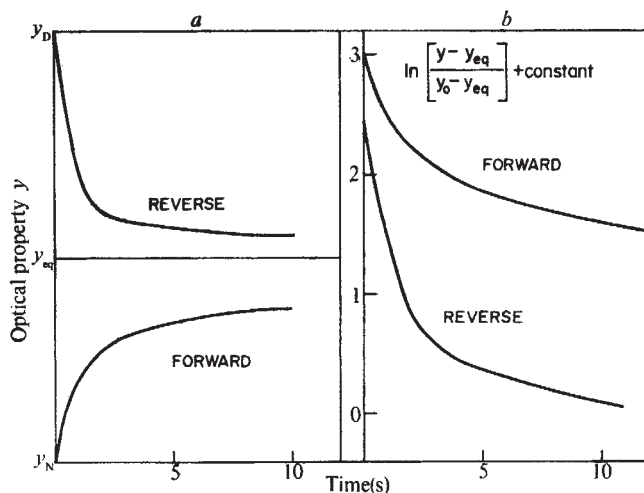
$$\frac{y - y_{eq}}{y_0 - y_{eq}} = e^{-\lambda t} \quad (1)$$



**Fig. 1** *a*, Reaction profile for the denaturation and renaturation of lysozyme in 2.5 M guanidine hydrochloride, pH 2.6, 25° C. The parameter  $y$  refers to absorbance at 301 nm,  $y_N$  and  $y_D$  representing the values of the native and fully denatured protein, respectively, and  $y_{eq}$  the equilibrium value (the equilibrium constant D/N being equal to 1 in these conditions). *b*, The corresponding semi-logarithmic plots, obeying equation (1).

where  $y_0$  is the value of  $y$  at  $t=0$  and  $y_{eq}$  is the equilibrium value of  $y$  or the value at  $t = \infty$ . The apparent rate constant  $\lambda$  is the same whether one starts from the native or denatured state, and is equal to the sum of the microscopic rate constants for the reactions  $N \rightarrow D$  and  $D \rightarrow N$  (Fig. 1).

We have during the past several years investigated several more complicated transitions, characterized by biphasic first order kinetic plots (Fig. 2). Such results necessarily imply that some species other than N and D accumulate to a significant extent during the reaction, and it is reasonable to suppose that this is an intermediate state on the pathway from N to D—a partially unfolded form of the protein molecule.



**Fig. 2** *a*, Reaction profile for the denaturation and renaturation of cytochrome c in 2.5 M guanidine hydrochloride, pH 6.5, 25° C. In this case  $y$  refers to absorbance at 400 nm, and the apparent equilibrium constant (see text) is equal to 0.9. *b*, The corresponding semi-logarithmic plot, obeying equation (3) with  $\lambda_1 = 1.2 \text{ s}^{-1}$ ,  $\lambda_2 = 0.06 \text{ s}^{-1}$ ,  $P_{1F} = 0.65$  and  $P_{1R} = 0.85$ . A stopped flow apparatus was used to follow the extent of reaction. One part of a protein solution in 0.1 M KCl or in 4.0 M guanidine hydrochloride was mixed with seven parts of a solution of guanidine hydrochloride at such concentration as to make the final concentration of guanidine hydrochloride 2.5 M.

The purpose of this article is to show that this conclusion is incompatible with our experimental results, those in Fig. 2 and in other studies of this kind. In other words, we shall show that the mechanism



in which X is a molecule with optical properties intermediate between those of N and D, cannot lead to a kinetic plot of the type shown in Fig. 2. It is true that the introduction of additional intermediates on the direct pathway in a mechanism such as



with monotonic change in optical properties ( $X_1$  closer to N and  $X_2$  closer to D) can formally reproduce the observed results, but the restrictions then imposed on the equilibrium constants and optical parameters are so severe that we have been forced to discard mechanism (II) for all our experimental studies, chiefly on the basis of incompatibility between these parameters at different concentrations of guanidine hydrochloride.

We have found, on the other hand, that a kinetic plot like that in Fig. 2 can be obtained by the introduction of a partially folded intermediate which cannot be transformed to N without first being unfolded. Physically, such an intermediate would represent an incorrectly folded conformation of the protein molecule. The simplest mechanism of this kind is represented by



It may be noted that mechanism (II) would also give correct results over a wide range of physical parameters if  $X_2$  were endowed with optical properties close to N and  $X_1$  with optical properties close to D, but this would similarly imply that the first product formed on renaturation has to be at least partially unfolded again before the conformation can return to the native state.

We shall now present a rigorous proof that mechanism (I) necessarily leads to a kinetic result different from that observed, and we shall show by a single example that mechanism (III) can give the correct result. More detailed discussion of the overall problem will be presented later.

The general solution of the kinetics of mechanism (I)<sup>2</sup> shows that the time dependence of the concentration of any of the three species can be expressed in terms of two exponential factors,  $e^{-\lambda_1 t}$  and  $e^{-\lambda_2 t}$ , where  $\lambda_1$  and  $\lambda_2$  are unique functions of the microscopic rate constants, given by the solutions of the quadratic equation,

$$f(\lambda) = \lambda^2 - \lambda \Sigma k_{ij} + (k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32}) = 0 \quad (2)$$

where  $\Sigma k_{ij}$  represents the sum of the four microscopic rate constants. The same values of  $\lambda$  apply to each species, and they are independent of the initial conditions: these factors enter only into the coefficients by which the exponential terms are multiplied.

We are interested in an optical property  $y$  which has values of  $y_N$ ,  $y_X$  and  $y_D$ , respectively, for the pure components at unit concentration. The time dependence of  $y$  is obtained from the equations for the concentrations of the three species, each multiplied by its appropriate  $y_i$ . The result can be written in terms of the same normalized parameter as was used in equation (1).

$$\frac{y - y_{eq}}{y_0 - y_{eq}} = P_1 e^{-\lambda_1 t} + P_2 e^{-\lambda_2 t} \quad (3)$$

$$\text{with} \quad P_1 + P_2 = 1 \quad (4)$$

When  $P_1$  and  $P_2$  are both positive, as has been the case for all experimental data we have fitted to equation (3), they may be regarded as the fractions of the total optical change associated with each exponential term. The equations do not, however, require  $P_1$  and  $P_2$  both to be positive. One of them can be

negative and the other greater than unity, the only requirement being that equation (4) is obeyed.

The expressions for  $P_1$  and  $P_2$  depend on the initial conditions and on the values of  $y_N$ ,  $y_X$  and  $y_D$ . We have obtained the solutions for two initial states, one in which the protein is initially entirely in the native state (we have called this the forward reaction, designated by subscript F), the other with the protein initially entirely in the fully denatured state (we have called this the reverse reaction, designated by subscript R). Because  $y_N$  and  $y_D$  are experimentally determinable, but  $y_X$  is not, the latter must be treated as an unknown parameter. This is most conveniently done in terms of a parameter  $\alpha$ , defined as

$$\alpha = \frac{y_X - y_N}{y_D - y_N} \quad (5)$$

If the properties of X are intermediate between those of N and D,  $0 < \alpha < 1$ . The final expressions for the coefficients are

$$P_{1F} = 1 - P_{2F} = \left[ \frac{1 + K_{app}}{K_{app}} \right] \frac{k_{12}}{\lambda_1 (\lambda_2 - \lambda_1)} \left[ \alpha (k_{32} - \lambda_1) + k_{23} \right] \quad (6)$$

$$P_{1R} = 1 - P_{2R} = (1 + K_{app}) \frac{k_{32}}{\lambda_1 (\lambda_1 - \lambda_2)} \left[ \alpha (k_{12} - \lambda_1) + (\lambda_1 - k_{12} - k_{21}) \right] \quad (7)$$

where  $\lambda_1 > \lambda_2$  and  $K_{app}$  is the apparent equilibrium constant previously defined<sup>1</sup> as

$$K_{app} = (y_{eq} - y_N)/(y_D - y_{eq}) \quad (8)$$

For a two-state process, involving only N and D,  $K_{app}$  is equal to the true equilibrium constant D/N.

The quantity of particular interest here is the ratio,  $P_{1F}/P_{1R}$ . By use of equation (2) it can be seen that  $k_{23}/(k_{32} - \lambda_1) = (\lambda_1 - k_{12} - k_{21})/(k_{12} - \lambda_1)$  and with this substitution one obtains

$$\frac{P_{1F}}{P_{1R}} = - \frac{1}{K_{app}} \frac{k_{12} (k_{32} - \lambda_1)}{k_{32} (k_{12} - \lambda_1)} \quad (9)$$

It is readily shown that  $\lambda_1$ , which is the larger of the two solutions of equation (2), must be larger than any of the microscopic rate constants  $k_{ij}$ . We first note from equation (2) that  $\lambda_1 > \frac{1}{2} \Sigma k_{ij}$  and  $\lambda_2 < \frac{1}{2} \Sigma k_{ij}$ . One of the  $k_{ij}$  is then designated as the largest of the microscopic rate constants (called  $k_{max}$ ), and it can be seen that  $\lambda_1 \neq k_{max}$  since  $f(k_{max})$  as given by equation (2) is always negative, regardless of which  $k_{ij}$  is the largest. Since  $df(\lambda)/d\lambda = 2\lambda - \Sigma k_{ij}$  it is clearly positive for  $\lambda_1$  and negative for  $\lambda_2$ . Thus, to satisfy equation (2),  $\lambda_1$  must be larger than  $k_{max}$  and  $\lambda_2$  must be smaller. In equation (9) both  $k_{32} - \lambda_1$  and  $k_{12} - \lambda_1$  are thus necessarily negative quantities and their ratio is positive.

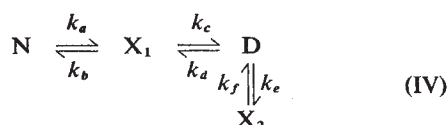
$K_{app}$  is also a positive quantity. It is theoretically possible that  $K_{app}$  as defined by equation (8) can be negative (if  $y_X$ , instead of lying between  $y_N$  and  $y_D$ , is less than either one), but experimentally it has always proved to be positive for the denaturation of proteins by guanidine hydrochloride, urea or by heating. It is therefore evident that equation (9) predicts that mechanism (I) will be characterized by a negative value for  $P_{1F}/P_{1R}$ . This means that if  $P_{1F}$  and  $P_{2F}$  are both positive,  $P_{1R}$  must be negative and  $P_{2R} > 1$ , and vice versa. The reaction profiles which would then be obtained are shown in Fig. 3. Because this prediction is contrary to the experimental result (Fig. 2), which is characterized by positive values for all four coefficients,  $P_{1F}$ ,  $P_{2F}$ ,  $P_{1R}$ ,  $P_{2R}$ , mechanism (I) is clearly inapplicable. The result is independent of the value of the



unknown parameter  $\alpha$ , with the proviso that negative values of  $\alpha$  leading to negative values of  $K_{app}$  are excluded on the basis of the experimental equilibrium studies.

The limitation imposed on  $P_{1F}/P_{1R}$  by mechanism (I) does not apply to mechanism (III). An example, giving reaction profiles of the right general type, is shown in Fig. 4.

Although mechanism (III) can lead to reaction profiles of the right general type, it cannot reproduce the specific data for the cytochrome c reaction (Fig. 2). This is because though mechanism (III) can lead to positive values for both  $P_{1F}$  and  $P_{1R}$  it cannot lead to a result in which both  $P_{1F}$  and  $P_{1R}$  lie between 0.5 and 1.0, as is true for the data of Fig. 2. (In the example provided by Fig. 4,  $P_{1F}=0.21$  and  $P_{1R}=0.94$ .) No three species mechanism can in fact account for the experimental result for cytochrome c, so that a second intermediate species must be introduced. As previously stated, a mechanism with two intermediates on the direct pathway from N to D can be excluded. But a mechanism such as



with one intermediate on the direct pathway, and one incorrectly folded form, is consistent with the results of Fig. 2 and with data for the cytochrome c reaction at other concentrations of guanidine hydrochloride. Such a mechanism will in general lead to a triphasic kinetic plot (three terms on the right hand side of equation (3)), but this will reduce to a biphasic plot if two of the three  $\lambda$ s have similar values. Mechanism (IV) will reproduce the experimental results of Fig. 2 if  $\alpha=0.7$  for  $\text{X}_1$  and 0.3 for  $\text{X}_2$ , with the following values for the rate constants:  $k_a=0.51 \text{ s}^{-1}$ ,  $k_b=0.69 \text{ s}^{-1}$ ,  $k_c=0.10 \text{ s}^{-1}$ ,  $k_d=0.06 \text{ s}^{-1}$ ,  $k_e=0.42 \text{ s}^{-1}$  and  $k_f=0.78 \text{ s}^{-1}$ .

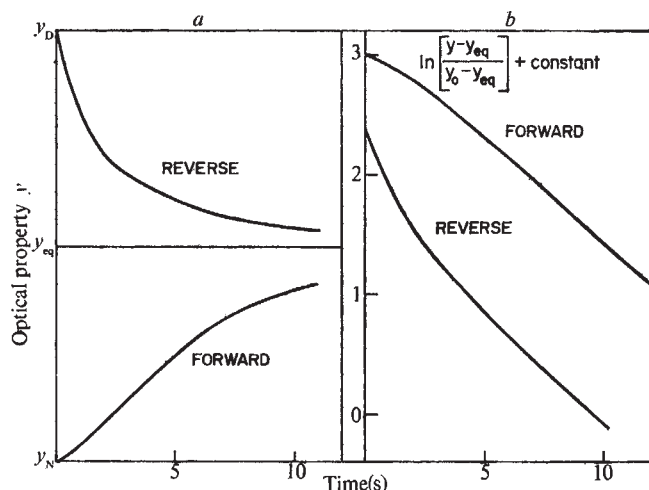


Fig. 3 a, Theoretical reaction profile for mechanism (I), with  $k_{12}=0.15 \text{ s}^{-1}$ ,  $k_{21}=0.013 \text{ s}^{-1}$ ,  $k_{23}=0.5 \text{ s}^{-1}$ ,  $k_{32}=0.3 \text{ s}^{-1}$ , and  $\alpha=0.1$ . The value of  $K_{app}$  is 1. b, Corresponding semi-logarithmic plots, obeying equation (3) with  $\lambda_1=0.904 \text{ s}^{-1}$ ,  $\lambda_2=0.176 \text{ s}^{-1}$ ,  $P_{1F}=-0.20$  and  $P_{1R}=0.50$ .

A description of the data of Fig. 2 in terms of mechanism (IV) requires that  $k_f > k_d$ . Similarly, to obtain Fig. 4 from mechanism (III) we had to set  $k_{32} > k_{31}$ . In both cases, the product formed most rapidly in the initial stages of renaturation of the fully denatured protein would be the incorrectly folded intermediate.

Similar considerations apply to the denaturation and renaturation of  $\beta$ -lactoglobulin in guanidine hydrochloride solution, which also does not obey simple first order kinetics as given by equation (1). The results of Simpson and Kauzmann<sup>3</sup> for the urea denaturation of ovalbumin are of the same type, and thus seem to exclude the sequential mechanism proposed by Kauzmann<sup>4</sup>. Schechter *et al.*<sup>5</sup> have shown that the kinetics of the refolding of pH-denatured staphylococcal nuclease can be described by equation (3) with  $P_{1R}$  and  $P_{2R}$  both positive. They do not report results in the forward direction, and so no definite conclusion can be reached: if the reaction profile for denaturation resembles that for renaturation their conclusion that the results indicate the occurrence of sequential first order processes would be incorrect.

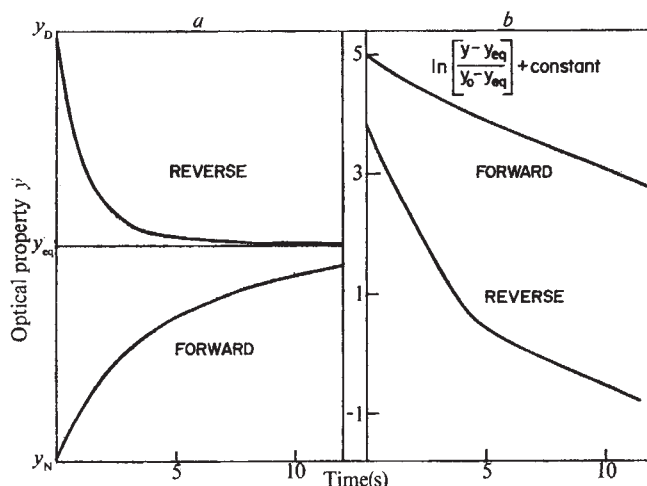


Fig. 4 a, Theoretical reaction profile for mechanism (III), with  $k_{13}=0.16 \text{ s}^{-1}$ ,  $k_{31}=0.054 \text{ s}^{-1}$ ,  $k_{23}=0.32 \text{ s}^{-1}$ ,  $k_{32}=0.54 \text{ s}^{-1}$ , and  $\alpha=0.3$ . The value of  $K_{app}$  is 1. b, Corresponding semi-logarithmic plots, obeying equation (3) with  $\lambda_1=0.904 \text{ s}^{-1}$ ,  $\lambda_2=0.173 \text{ s}^{-1}$ ,  $P_{1F}=0.21$  and  $P_{1R}=0.94$ .

Incorrectly folded intermediate states could be a general feature of the time course of protein denaturation processes. Such a finding would suggest that the initial steps in the folding of a structureless polypeptide chain are rapidly reversible and do not influence the ultimate result. It would be consistent with the fact that most proteins return to the true native conformation after *in vitro* unfolding, in conditions where folding may begin at any part of the unfolded polypeptide chain, whereas the original *in vivo* folding presumably must begin near the amino-terminal end of the polypeptide chain.

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# LETTERS TO NATURE

## PHYSICAL SCIENCES

### Search for Optical Circular Polarization in the Crab Nebula

It has recently been suggested by Gunn and Ostriker<sup>1</sup> and by Rees (paper presented at the IAU symposium 46 on the Crab Nebula, Manchester, August 1970) that the magnetic field in the Crab Nebula which causes relativistic particles to emit synchrotron radiation may be an oscillating 30 Hz electromagnetic field from the pulsar rather than a static field. Rees has shown that if this is the case, the nebula should show a component of circular polarization of order a few per cent in visible light. This is predicted to have opposite sense in the NW and SE regions of the nebula, supposing that the spin axis of the pulsar is in the direction of linear polarization.

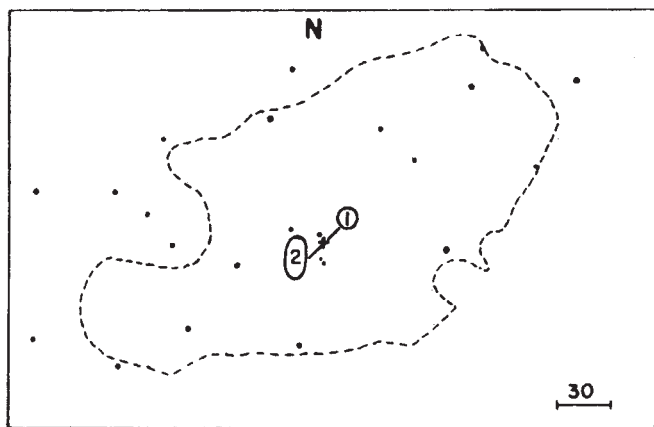


Fig. 1 The Crab Nebula, showing the outline of the nebula, the principal stars, and the two regions where the circular polarization of the nebula was measured (labelled 1 and 2). The line joining the two regions, and passing through the pulsar (indicated by a cross), is the approximate direction of the linear polarization of the nebula at the pulsar<sup>3</sup>.

This letter reports an attempt to detect the predicted circular polarization. We observed the Crab Nebula on November 4 and 6, 1970, with the polarimeter described by Angel and Landstreet<sup>2</sup> on the 82 inch telescope at McDonald Observatory. No filters were used, resulting in a pass band of roughly 4000–5800 Å, and the Pockels cell was set to give a quarter wave retardation at  $\lambda 4600$ . The polarimeter has a slight sensitivity to linear polarization when it is being used to measure circular polarization, with an apparent effect equal to about 2% of the linear component. To eliminate the effect due to the strong linear polarization of the Crab Nebula, alternate observations were made with the polarimeter in its normal position on the telescope, and rotated by 90° about the optical axis. The rotation does not affect the signal due to true circular polarization, but spurious signals induced

by linear polarization reverse sign and are eliminated when an average is taken of measurements in the two orientations.

Measurements were made of the two regions of the Crab Nebula indicated in Fig. 1. Region 1 is centred at  $\Delta\alpha = +1^{\circ}2$  and  $\Delta\delta = -10''$  from the position of the pulsar, and region 2 is at  $\Delta\alpha = -0^{\circ}8$  and  $\Delta\delta = +13''$ . A 7.2 inch diaphragm was used, but because of poor seeing and telescope drift the measured polarizations are averages over the regions indicated. No significant polarization was detected, the measured values after corrections for the night sky background being  $+0.024 \pm 0.040\%$  in region 1 and  $+0.034 \pm 0.047\%$  in region 2. The quoted standard deviations arise from counting statistics. It is apparent that if any circular polarization is present in these regions, it is substantially smaller than that expected from Rees's theory.

We thank Professor Brian Warner and Mr Edward Nather for cooperation, and the director of McDonald Observatory for making the 82 inch telescope available to us. This work was supported by grants from the Research Corporation, the National Research Council of Canada, and by the US National Aeronautics and Space Administration.

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Editor's note: Next Monday's *Nature Physical Science* will contain an article by Rees discussing the polarization of the Crab Nebula.

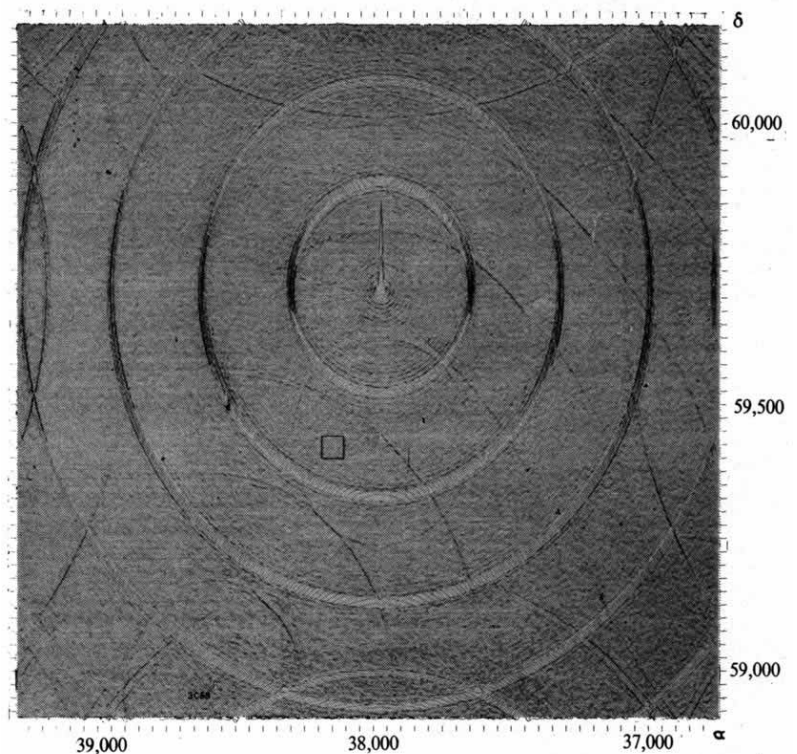
### Absence of Radio Emission from Maffei I

SPINRAD *et al.*<sup>1</sup> have suggested that the infrared object discovered by Maffei<sup>2</sup> during a search for T Tauri variables is the nucleus of an exceptionally close giant elliptical galaxy, the main body of which is obscured by absorption in our galaxy. Though the observations point strongly in this direction, the suggestion is nevertheless a considerable extrapolation from the actual observations and it is therefore important to try to get additional information on the properties of the object.

We have made a 12 h observation of a field surrounding Maffei I with the synthesis radio telescope at Westerbork to look for continuous radiation at 1,415 MHz. As our diagrams show, the result was negative. With the noise figure of about  $\pm 0.0012$  flux units attained, this means that the upper limit



**Fig. 1** Synthesized picture of  $1.3^\circ \times 1.3^\circ$  after 12 h of observing. Right ascension and declination are indicated in decimal degrees. The intensity scale is such that  $0.1^\circ$  in declination corresponds to 0.2 flux units. Twenty baselines were used, ranging from 198 to 1,566 m in steps of 72 m. Grating rings are seen at multiples of  $10'$  arc around each source. These are due to the regular interval of 72 m in the distribution of interferometer spacings. After a complete synthesis of four periods of 12 h of observing the first grating ring would have a radius of  $40'$ . The synthesized half power beamwidth in this picture is  $22''$  arc in right ascension and  $43''$  in declination. The strongest source in the field is a previously unknown point source with a flux density of 0.427 flux units (after correction for the antenna pattern of the interferometer elements). The location of the optical position of Maffei I is indicated by the box. The source 3C69 is seen at the bottom left. The attenuation for this source by the primary antenna pattern is about 13 dB. According to Macdonald, Kenderdine and Neville<sup>6</sup>, 3C69 is a double source with integrated fluxes of 1.53 and 21.4 flux units at 1,407 MHz. Our observations are in very good agreement with these values.



of the radiation of a point source at the centre of Maffei I is about 0.0025 flux units. We have made a second Fourier transform with an effective beamwidth of slightly less than  $2'$ . If the radiation were a point source for this beam, the upper limit would be about 0.0006 flux units. At a distance of 1 megaparsec as estimated by Spinrad *et al.*, these two upper limits correspond to an upper limit for the total radio energy  $L$  of  $5 \times 10^{34}$  erg  $s^{-1}$  for a nuclear source and  $1.2 \times 10^{34}$  for a nuclear region of 500 pc diameter, if the radiospectrum is supposed to vary as  $\nu^{-0.75}$  between  $10^7$  and  $10^{11}$  Hz and to break off at  $10^{11}$  Hz.

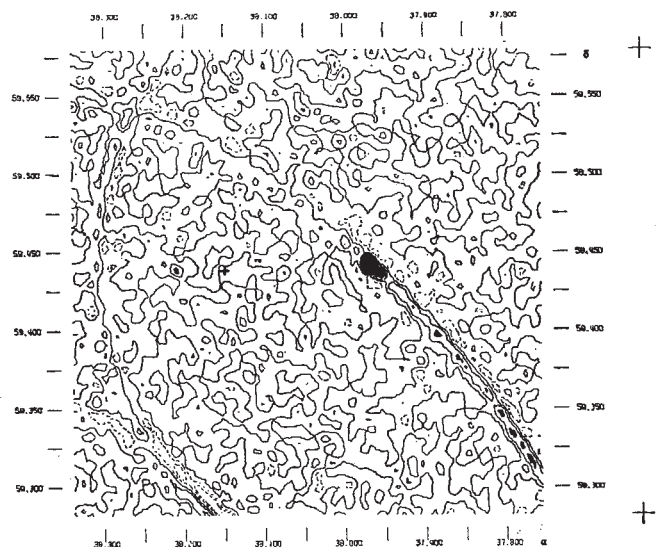
The upper limit for the radiation of Maffei I is  $10^4$  times less than the lower limit of radiation detected up to now from other giant elliptical galaxies. But the number of giant ellipticals that have been detected is only a relatively small fraction of the objects observed, and it may be that some giant ellipticals have a very much lower radio luminosity.

The two principal observing programmes on elliptical galaxies are by Rogstad and Ekers<sup>3</sup> and by Heeschen<sup>4</sup>. The former observed all E and So galaxies for which radial velocities have been published by Humason, Mayall and Sandage<sup>5</sup>, so that rough distances can be estimated. Galaxies in the Virgo cluster were excluded from this programme. Heeschen observed all E and So galaxies brighter than photographic magnitude 11.2 that could be reached from Green Bank. Both series of observations were made at 11 cm wavelength. Heeschen states that his limiting brightness was 0.05 flux units (1 flux unit =  $10^{-26}$  W  $m^{-2}$  Hz $^{-1}$ ). In the catalogue of Rogstad and Ekers the lowest flux density among the fourteen galaxies which they label as "positively detected" is 0.20 flux units.

If we define a giant elliptical galaxy as having a photographic absolute magnitude  $-19.0$  or brighter, there are eighty-three giant Es in Rogstad and Ekers's list. Eight of these, or 10%, are indicated as "positively detected". Their  $\log L$  values range from 39.4 to 41.4, and average 40.3, that is,  $10^6$  times higher than the upper limit for Maffei I. Of the remaining seventy-five Rogstad and Ekers indicate possible fluxes for thirty-five, which, if real, would correspond with an average  $\log L$  of 39.5. But all of these fluxes are very close to the sensitivity limit of the survey, and it seems likely that most of them are fortuitous coincidences. It seems safer to consider them as upper limits to the true fluxes. The average upper limit

of  $\log L$  for the seventy-five objects is then 39.2, with a relatively small spread; there is none below 38.4.

Heeschen's survey is complete to a somewhat lower flux density. He detected radio emission from eleven out of fifty objects, or 22%, as against 10% in Rogstad and Ekers's list. The values of  $\log L$  for the giant ellipticals range from 38.8 to 41.7, with an average of 40.0. For nine of the detected galaxies Heeschen's observations also provide information on the distribution of the radiation; in seven a large portion of the radiation was observed to be concentrated in a small nucleus, in the two other galaxies only a halo was detected.



**Fig. 2** Contour plot of the region around the optical position of Maffei I, which is indicated by the cross. The strong source at the centre of the field has been removed from the map. The contour values are  $-5$ ,  $-2.5$ ,  $0$ ,  $2.5$ ,  $5$ ,  $10$ ,  $20$  and  $40$  milliflux units. Negative contours are dashed. Regions above 5 milliflux units are shaded. In the picture the grating ring of 3C69 is seen, together with a weak source and part of its first (negative) grating ring.

Although this discussion shows that no giant elliptical galaxy is known to have a radio luminosity smaller than  $5 \times 10^{38}$ , the available data are evidently insufficient to exclude the possibility of the existence of giant ellipticals with radio luminosities of the order of  $10^{34}$ . At present we can only state that Maffei I is at least four orders of magnitude fainter in radio luminosity than the faintest giant ellipticals observed.

A programme of flux measures with a beam of about  $30''$  and much lower sensitivity limits than the surveys quoted is now being observed at Westerbork. After the completion of that programme one may be able to say definitely whether or not Maffei I differs radically from other giant ellipticals in its radiation at radio frequencies.

For this work the observations were prepared by Raimond, the programming is due to Brouw, the positioning and identification on the maps were done by Van der Kruit, and I thank Van der Laan for taking part in the discussion.

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*Sterrewacht te Leiden*

Received January 13, 1971.

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<sup>5</sup> Humason, M. L., Mayall, N. U., and Sandage, A. R., *Astron. J.*, **61**, 97 (1956).

<sup>6</sup> Macdonald, G. H., Kenderdine, S., and Neville, Ann C., *Mon. Not. Roy. Astron. Soc.*, **138**, 259 (1968).

## Amino-acids, Aliphatic and Aromatic Hydrocarbons in the Murchison Meteorite

Two recently fallen carbonaceous chondrites have provided organic analytical results significantly different from those obtained with other carbonaceous chondrites<sup>1-3</sup>. The Allende meteorite, a type III carbonaceous chondrite which contains 0.27% carbon and 0.007% nitrogen<sup>4</sup>, was shown to have only traces of extractable organic compounds<sup>5</sup>. Small amounts of aliphatic and aromatic hydrocarbons were released by heating while no significant amounts of nitrogen containing compounds were detected<sup>6-8</sup>. This is consistent with the extremely low nitrogen content of this carbonaceous chondrite. Another unique feature of this meteorite is the heterogeneous distribution of certain carbon-containing inclusions<sup>9</sup>. The Murchison meteorite, a type III carbonaceous chondrite, contains substantial amounts of amino-acids, predominantly cyclic aliphatic hydrocarbons<sup>10</sup>, which are consistent with the appreciable content of carbon and nitrogen of this meteorite (2 and 0.16% respectively). The amino-acids include non-protein components such as sarcosine and 2-methylalanine and, significantly, approximately racemic mixtures of D and L enantiomers of protein amino-acids such as alanine, valine and proline<sup>10</sup>.

The presence of D and L enantiomers of protein amino-acids, and aspartic acid, has been observed before in the fraction containing the bound amino-acids of the Murray meteorite<sup>11</sup>. The predominance of the L configuration observed in this case, and a comparison with results obtained with soil hydrolysates, suggested that these amino-acids were derived essentially from terrestrial contamination<sup>11</sup>, or that only a small percentage of them (less than 10% on average) were probably indigenous meteoritic amino-acids of a racemic composition<sup>12</sup>. A racemic composition was also indicated for the smaller amounts of supposedly free amino-acids (hydrolysed water extract) present in the Murray<sup>12</sup>. In a continuation of these studies we now have analysed the organic compounds of the

Murchison meteorite by water extraction and combined gas chromatography and mass spectrometry.

A relatively large piece of the Murchison meteorite almost entirely covered with fusion crust was the primary source of material for this study. It had no visible fractures and a minimum of soil-staining and weathering, giving a very clean general appearance. Several inside pieces were taken after removing more than two centimetres of the fusion crust and outer surface from the specimen. One of the selected inside pieces was pulverized to less than 60 mesh size and processed for amino-acid analysis. About 20 g of this material was refluxed with 25 ml. of triply distilled water for 14 h. After decanting, filtering and washing, the meteorite residue was re-extracted for 1 h and all extracts were combined and evaporated to dryness in a current of pure nitrogen. The residue from the water extract was dissolved in 10 ml. of 6 M HCl and hydrolysed for 24 h *in vacuo* in a sealed tube at 100° C. After evaporation to dryness the hydrolysate was dissolved in water and desalted with 'Dowex 50' (H<sup>+</sup>) ion exchange resin and eluted with water and 2 N NH<sub>4</sub>OH (10 × bed volume). The eluate was evaporated as described and the residual amino-acids were esterified with 3 M HCl-isopropanol at 100° C for 30 min and then converted into N-trifluoroacetyl-amino-acid-isopropyl esters<sup>13,14</sup>. Aliquots (in CHCl<sub>3</sub>) of these derivatives were analysed by combined gas chromatography and mass spectrometry and by gas chromatography on optically active phases using methods described before<sup>15,16</sup>.

Fig. 1A shows a representative gas chromatogram obtained by injection of 1 µl. of the N-TFA isopropyl esters into a 150 m × 0.5 mm interior diameter capillary gas chromatographic column coated with 3% SF-96 in an LKB 9000 gas chromatograph-mass spectrometer. This column, which has an efficiency of 180,000 plates, was also used for the analysis of the hydrocarbons. Preliminary mass spectrometric evidence was obtained for the presence of glycine, alanine, 2-methylalanine, amino-butyric acid, valine, glutamic acid, proline and some of the leucines. Glycine was the principal amino-acid observed (5.3 µg/g) and the others were found in amounts of the order of 0.1 µg/g or more (alanine 3.1 µg/g). An appreciable number of other amino-acids, and possibly amines, remain to be identified as shown by the relatively large number of components of the gas chromatogram. More than half of the major peaks have been identified, however, and most agree with the observations by Kvenvolden *et al.*<sup>10</sup>. Gas chromatographic analysis of the N-TFA-amino-acid-isopropyl esters on a 162 m long × 0.5 mm interior diameter capillary column coated with N-TFA-L-valyl-L-valine cyclohexyl ester gave evidence for the separation of D and L enantiomers of alanine and proline in approximately equimolar amounts. This provides evidence for the chemical formation of amino-acids in one or several of the following ways: (a) extraterrestrial abiotic synthesis, before, during or after the formation of the meteorite parent body; (b) extensive diagenesis, in the unlikely event that the primary amino-acids were of either L or D configuration; (c) formation by synthetic or decomposition reactions during the entry of the meteorite through the terrestrial atmosphere, and (d) chemical synthesis from amino-acid precursors during the hydrolysis of the water extracts. Although (d) cannot yet be ruled out, the first mode of synthesis seems the most likely<sup>10-12</sup>.

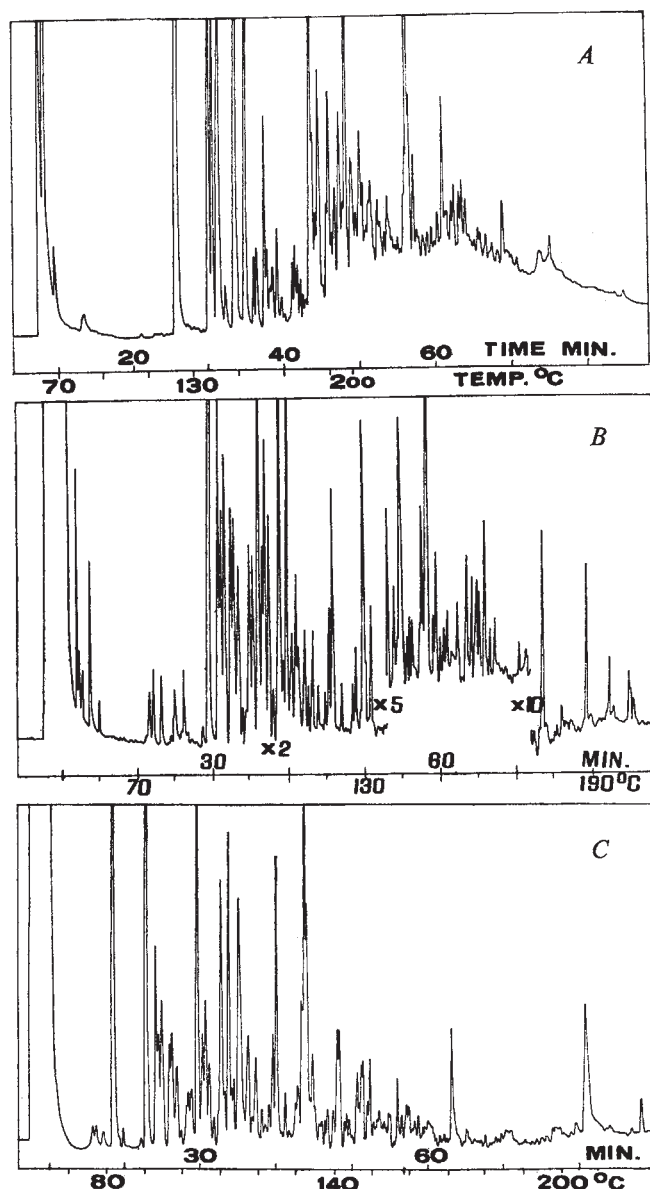
Another of the inside pieces of our specimen, weighing 7.5 g, was used for the analysis of hydrocarbons. It was washed with 10 ml. of a mixture of benzene and methanol (3:1), dried, pulverized and Soxhlet extracted with 15 ml. of the same mixture in a modified all glass apparatus<sup>17</sup>. After refluxing for 3 h the extract was concentrated to a few microlitres and fractionated on a silica gel column with pentane, benzene and methanol<sup>18</sup>. About a 1/10 aliquot of the pentane fraction concentrated to a few microlitres was injected into the SF-96 capillary column.

Fig. 1B shows a representative gas chromatogram of the pentane fraction. The aliphatic hydrocarbons observed



range from  $C_{10}$  to relatively high molecular weight components. The most abundant series corresponds to the branched saturated alkanes. They include predominantly monomethylated and dimethylated isomers. Intense ions that correspond to the loss of fragments with  $m/e$  29, 43, 57 and higher values suggest the presence of branching at positions 2, 3, 4 and so on, respectively.

The  $C_nH_{2n-1}$ ,  $C_nH_{2n-3}$  and  $C_nH_{2n-5}$  series were also observed but were substantially less abundant. Up to  $C_{15}$  the  $C_nH_{2n-1}$  ions seemed to belong to olefins rather than monocyclic alkanes, for the  $m/e$  doublets corresponding to



**Fig. 1** Gas chromatograms obtained on an LKB 9000 gas chromatograph-mass spectrometer with a 150 m  $\times$  0.5 mm stainless steel open tubular capillary column coated with SF-96 operated at 1.41 kg/cm<sup>2</sup> and the temperatures listed. *A*, *N*-TFA-isopropyl derivatives of the water extracted amino acids. Column isothermal at 70° C for 10 min and programmed at 3° up to 200° C. The nine major peaks of this chromatogram are, from left to right: solvent front, unknown; alanine, unknown; glycine, unknown; valine, norleucine (internal standard), unknown; and glutamic acid. Almost all other peaks, including the above listed unknowns, have fragmentation patterns of amino-compounds. *B*, Alkanes from the pentane fraction of the benzene-methanol extract. They range from nine to sixteen carbon atoms. Saturated structures predominate in this range. The column was isothermal at 50° C for 10 min and programmed at 2°/min up to 200° C. *C*, Benzene fraction from benzene/methanol extract. The column was isothermal at 65° C for 15 min and programmed at 2°/min to 200° C.

the fragment ions  $C_nH_{2n-1}$  and  $C_nH_{2n-2}$ , for  $n=5, 6$  or higher, characteristic of cyclics, were not present. The  $C_nH_{2n-3}$  series increased in intensity after the  $C_{14}$  region, and suggested bicyclic structures with aliphatic branching. Fig. 1C shows a representative gas chromatogram of the hydrocarbon fraction eluted with benzene. Mass spectra with fragmentation patterns corresponding to naphthalene, phenanthrene or anthracene, and branched aliphatic chains were observed. These data, together with gas chromatographic retention times, indicate the presence of alkyl substituted dicyclic and tricyclic aromatic compounds<sup>19</sup>. There is an intriguing similarity in the composition and complexity of these hydrocarbon mixtures with those obtained by pyrolysis and other experiments of synthesis (see, for example, ref. 20). Preliminary analytical results by vaporization pyrolysis gas chromatography-mass spectrometry<sup>8</sup> have also shown the presence of large amounts of aliphatic and aromatic hydrocarbons and other organic compounds with heteroatoms (unpublished results).

Overall, the Murchison meteorite contains substantial amounts of extractable organic compounds, which give distribution patterns significantly different and much more complex than those observed previously, indicating abiotic synthesis, extensive diagenesis and related chemical processes, or both. This carbonaceous chondrite seems to be one of the first examined where the amount of terrestrial biological contamination is very small.

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## Configuration of Amino-acids in Carbonaceous Chondrites and a Pre-Cambrian Chert

IN connexion with studies on the origin and evolution of life on Earth<sup>1</sup>, and its possible detection in space, we have developed a sensitive method for the analysis of protein amino-acids, which includes the simultaneous determination of their optical configuration<sup>2</sup>. The approach is based on the separation of enantiomeric *N*-trifluoroacetyl (*N*-TFA)-isopropyl esters by gas chromatography with an optically active stationary phase<sup>3</sup>. We wish to describe here the application of the method to meteorites and a Pre-Cambrian sediment.

The occurrence of traces of amino-acids in carbonaceous chondrites<sup>4-8</sup> has been ascribed to contamination and abiogenesis. But the determination of the configuration of these compounds seems to permit differentiation between these two possibilities. Amino-acids found in terrestrial living organisms are almost exclusively of the *L* configuration; in fossil material, however, the free or bound amino-acids slowly racemize. From data on the configuration of amino-acids from the interior of shells, Hare and Abelson<sup>9</sup> estimated a racemization period of about 10<sup>5</sup> yr. Recent results from our laboratory on tar covered bones from the Rancho La Brea, Los Angeles, show significant racemization after 12,000 yr. Thus, an *L* configuration of the amino-acids in meteorites would be evidence for contamination, whereas the presence of racemates would argue for chemical synthesis or for very old biogenic amino-acids.

We extracted samples (weighing 2 g) of Orgueil, Mokoia, and Murray meteorites, previously treated with organic solvents<sup>10</sup>, with water and then hydrolysed at 100° C with 6 M HCl for 24 h. After filtration the hydrolysate was evaporated to dryness. We dissolved each residue in 2 ml. of distilled water, desalted on 'Dowex 50' (20 ml.), and the amino-acids were eluted with 500 ml. of 2 M NH<sub>4</sub>OH. After evaporation to dryness the residual amino-acids were transformed into *N*-TFA-isopropyl esters as described in ref. 2. Aliquots (in CHCl<sub>3</sub>) were chromatographed at 110° C on a 500 foot × 0.02 inch stainless steel capillary column, coated with *N*-TFA-L-valyl-L-valine cyclohexyl ester<sup>2</sup>, using He as the carrier gas at 20 pounds/inch<sup>2</sup> and an FID. Two additional specimens of Murray (3.3 g and 4.6 g) which had not been treated with organic solvents were Soxhlet extracted with water for several

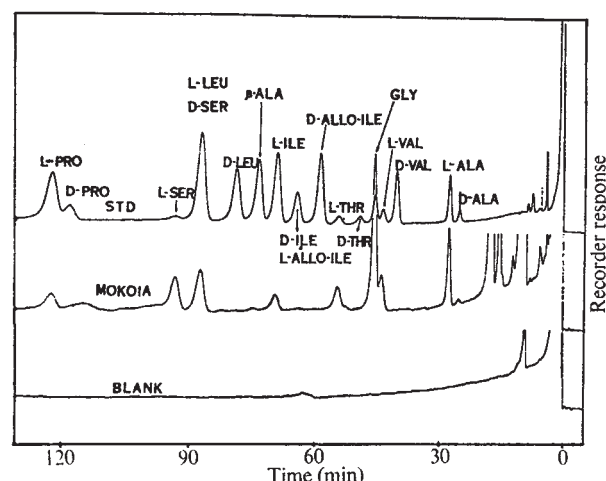


Fig. 2 Mokoia meteorite. Chromatogram of the *N*-TFA-isopropyl esters of the amino-acids from acid hydrolysis of the organic solvent and water extracted meteorite residue.

hours and the water extracts hydrolysed and analysed in the same manner.

Chromatograms for Orgueil, Mokoia, and Murray meteorites are given in Figs. 1-3. We identified the peaks by comparison with standards and, for the Murray only, by GC-mass spectrometry<sup>11</sup>. Published data<sup>4-6</sup> on amino-acids in Orgueil are in agreement with the present results.

Table 1 Percentage of D-Enantiomers in the Amino-acids from Hydrolysates of Carbonaceous Chondrites and a Soil Sample

| Amino-acid                    | D/D + L × 100 |        |        |        |
|-------------------------------|---------------|--------|--------|--------|
|                               | Orgueil       | Mokoia | Murray | Soil * |
| Alanine                       | 22.5          | 6.5    | 13.0   | 8.6    |
| Valine                        | 0.0†          | 0.0†   | 2.4    | 0.5    |
| Threonine                     | 0.0†          | 0.0†   | 0.0†   | 0.0†   |
| D-Alloisoleucine-L-isoleucine | ‡             | 0.0†   | 3.8§   | 1.3    |
| Leucine                       | 0.0†          | 0.0†   | ¶      | ¶      |
| Proline                       | 0.0†          | 0.0†   | 12.3   | 4.2    |
| Aspartic acid                 | 0.0†          | 0.0†   | 24.6   | 8.0    |

\* Taken 5-7 cm beneath surface in a grassy area of Memorial Park, Houston, Texas.

† Below limits of detection. A small amount of D-serine might be present in the L-leucine, since the peaks of the *N*-TFA-isopropyl esters of these two compounds overlap. For this same reason no data are given for the enantiomeric composition of serine.

‡ D-Alloisoleucine was added to this meteorite as an internal standard.

§ After correction for D-alloisoleucine, contained in added L-alloisoleucine (Fig. 3).

¶ On the assumption that the leucine peak contained a negligible amount of D-serine, the figures are 1.9% for Murray and 2.4% for the soil sample.

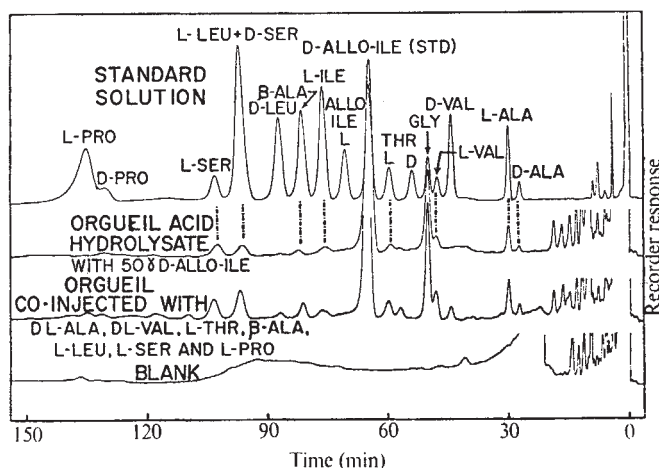
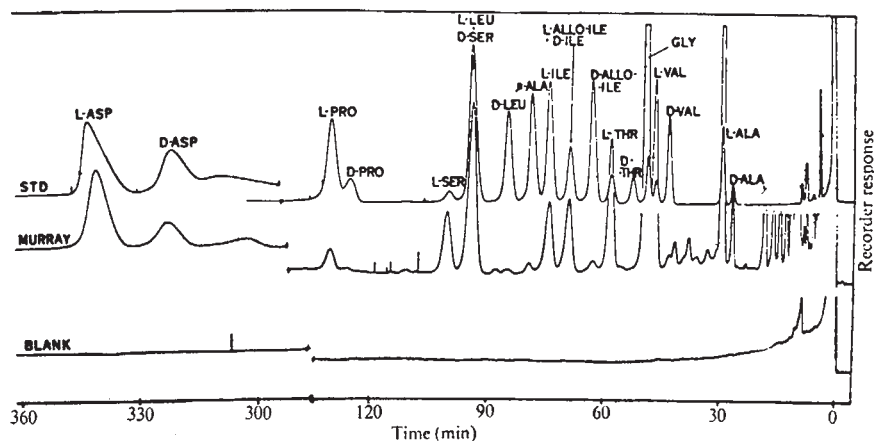


Fig. 1 Orgueil meteorite. Chromatogram of the *N*-TFA-isopropyl esters of the amino-acids from acid hydrolysis of the organic solvent and water extracted meteorite residue. D-Alloisoleucine was added to the meteorite as an internal standard. Taking the L-isoleucine peak in the second and third chromatogram as reference shows that the L-Val, L-Thr, β-Ala, L-Leu, and L-Ser peaks are increased on co-injection. The ratio of the alanine peaks is changed on co-injection of the DL standard, as expected. The small peak in the proline region does not coincide with the L-isomer (see co-injection), nor with the D-isomer (relative retention time).

These results suggest multiple sources for the amino-acids in carbonaceous chondrites. The data on Orgueil and Mokoia (Figs. 1 and 2; Table 1) show that the bound amino-acids in these meteorites are almost exclusively of the *L* configuration with the exception of D-alanine, which is present in amounts of 22.5% and 6.5%, respectively. Although it could be interpreted, on the basis of the exhaustive organic solvent and water washing of the meteorites, that the L-amino-acids are of extra-terrestrial origin, we believe that the bulk of the bound amino-acids are the result of terrestrial contamination by micro-organisms. The presence of some D-alanine is not against this conclusion, for this particular D isomer occurs in bacterial cell



Fig. 3 Murray meteorite. Chromatogram of the *N*-TFA-isopropyl esters of the amino-acids from acid hydrolysis of the organic solvent and water extracted meteorite residue. L-Alloisoleucine was added as an internal standard.



walls and other microbes. A minimum of 1,800 viable bacteria  $g^{-1}$  of Mokoia has been observed<sup>12</sup>, and non-viable micro-organisms have been estimated to be up to thirty times more abundant<sup>13</sup>.

Murray, on the other hand, had not only 13% D-alanine, but also 2.4% D-valine, 3.8% D-alloisoleucine and as much as 12.3% D-proline and 24.6% D-aspartic acid in the water insoluble or bound amino-acid fraction (Fig. 3; Table 1). A microbiological count gave 6,000 viable bacteria  $g^{-1}$  for this meteorite<sup>12</sup>, indicating considerable contamination. We therefore analysed a recent soil sample, which showed a similar pattern (Table 1) to that found for Murray. The figures for D-amino-acids in the soil are not as high as in the meteorite but they are significantly higher than the usual analytical racemization values<sup>14</sup>.

Considering the relatively larger amounts of D-amino-acids in Murray, compared with the soil (the difference is about 7% on average) and the presence in the Murray chromatogram (Fig. 3) of many minor additional peaks, it seems that a small percentage of the amino-acids of this meteorite are racemic mixtures of possible abiotic origin. This interpretation gains support from an analysis of the water extracts of Murray, where peaks with the retention time of D and L-alanine were observed in approximately equal quantities (44% and 56% respectively), the total amount of this amino-acid in the hydrolysed water extracts being only  $0.05 \mu g g^{-1}$  (unpublished observations). Final opinion should, however, be reserved until more information is available on the chemical synthesis of amino-acids from simple precursors during the entry of the meteorite through the atmosphere, or during the acid hydrolysis step, and on the configuration and inversion of amino-acids in soils. The presence of traces of  $\beta$ -alanine in Murray (Fig. 3) could also be an indication of abiogenesis, but it is known that this amino-acid can be formed by bacterial decarboxylation of aspartic acid<sup>15</sup>.

A wide range of amino-acids has been detected in Pre-Cambrian sediments. It was suggested<sup>16</sup> that these compounds were formed biogenically  $1-3 \times 10^9$  years ago. Abelson and Hare<sup>17</sup> have, however, demonstrated the L configuration of amino-acids from a Gunflint chert ( $1.9 \times 10^9$  yr old), using enzymatic and other tests. This conclusion has since been confirmed<sup>18-20</sup>.

A hydrolysate of the Fig Tree chert prepared in Dr Kvenvolden's laboratory<sup>18</sup> was analysed by gas chromatography with an asymmetric stationary phase in our laboratory. We found all the amino-acids reported earlier. With the exception of D-alanine, any peaks corresponding to D-isomers are negligible.

These results provide convincing evidence that the amino-acids in the Fig Tree chert are almost exclusively of the L configuration and argue for recent terrestrial contamination of the samples, as in the case of the Orgueil and the Mokoia meteorites. A similar argument can be made out for Murray, although in this case about 90% of the amino-acids probably

result from biological contamination and the remainder (both D and L configurations) are of probable chemical origin. It remains to be seen whether the small amounts of racemic amino-acids were syngenetic with the meteorite parent body or were synthesized later during the extraterrestrial or terrestrial history of Murray. The presence of racemic mixtures of several amino-acids in the Murchison meteorite has been reported recently<sup>21</sup>; we have confirmed this observation.

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## Miocene Volcanism in the North Sea

I HAVE been informed by an oil company that there is a formation of Miocene age in the central North Sea area which looks like volcanic tuff. I have made a preliminary examination of three specimens from this formation, which is about 100 m thick and widespread.

section contains a few tiny quartz grains and one feldspar grain (Fig. 1). The tuff looks like a partly devitrified glass irregularly made up of very small crystallites. One section contains curved pigment boundaries, possibly a trace of perlite texture. Other sections show a clearly sedimentary stratification, marked by thin black stringers rich in iron ore pigment. The brown tuff contains microfossils 0.1–0.01 mm in

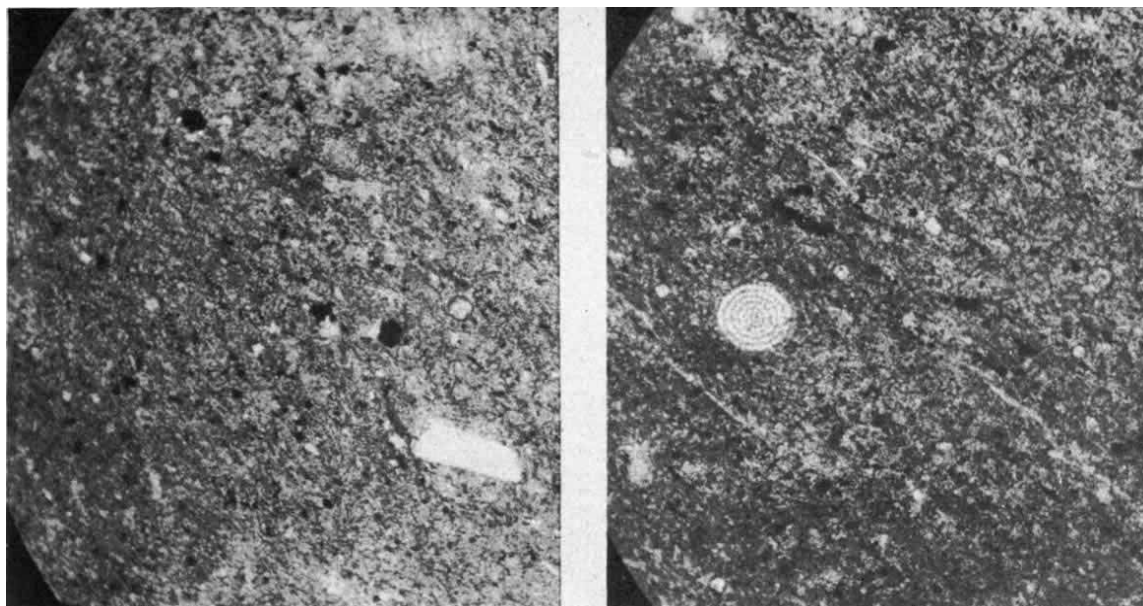


Fig. 1 Photomicrographs of North Sea tuff, each 0.7 mm long. On the left is tuff with feldspar crystal (specimen X). On the right is layered tuff with microfossil (specimen 5100-30).

The material consists of fragments varying from 10 mm across to the size of dust. The colour is light to medium grey, with some brownish tints. It looks rather homogeneous to the naked eye, but rather more grainy under a microscope. There are also some evenly distributed black grains between 0.1 and 0.2 mm across. These are rather spherical pyrite grains with a framboidal texture in polished section. Thin sections show that the rock is fine grained, with a medium to strong, brownish pigment of iron ore dust in an irregular distribution. One

size comprising both brownish shells and thin worm-like structures, embedded in a material which is mostly structureless, which suggests that the particle size of the embedding ash was less than 0.01 mm.

A careful refractometer study after heavy liquid separation confirms the existence of pyrite in the form of the larger grains, together with a little magnetite and goethite. Siderite spherules and amphibole crystals occur in the heavy fraction (>2.96). Biotite, quartz and feldspar (probably albite) were found in

Table 1 Analyses and Norms of Four Ashes from the Central North Sea

| Specimen                             | X     | % by weight<br>5100-30 | 5220-50<br>Light | 5220-50<br>Dark | X     | % by weight without water<br>5100-30 | 5220-50<br>Light | 5220-50<br>Dark |
|--------------------------------------|-------|------------------------|------------------|-----------------|-------|--------------------------------------|------------------|-----------------|
| SiO <sub>2</sub>                     | 62.70 | 62.00                  | 62.00            | 60.50           | 70.8  | 68.2                                 | 68.0             | 66.8            |
| TiO <sub>2</sub>                     | 0.85  | 0.92                   | 0.95             | 0.90            | 1.0   | 1.0                                  | 1.0              | 1.0             |
| Al <sub>2</sub> O <sub>3</sub>       | 13.40 | 13.20                  | 13.40            | 13.20           | 15.2  | 14.5                                 | 14.7             | 14.6            |
| Fe <sub>2</sub> O <sub>3</sub> (tot) | 1.54  | 7.80                   | 7.80             | 8.60            | 1.7   | 8.6                                  | 8.5              | 9.5             |
| MnO                                  | 0.05  | 0.05                   | 0.05             | 0.05            | 0.1   | 0.1                                  | 0.1              | 0.1             |
| MgO                                  | 1.80  | 1.90                   | 1.95             | 1.83            | 2.0   | 2.1                                  | 2.1              | 2.1             |
| CaO                                  | 5.18  | 1.55                   | 1.50             | 1.60            | 5.9   | 1.7                                  | 1.6              | 1.8             |
| Na <sub>2</sub> O                    | 1.00  | 1.30                   | 1.50             | 1.58            | 1.1   | 1.4                                  | 1.6              | 1.7             |
| K <sub>2</sub> O                     | 1.90  | 2.15                   | 2.20             | 2.20            | 2.2   | 2.4                                  | 2.4              | 2.4             |
| H <sub>2</sub> O+                    | 7.04  | 5.12                   | 5.23             | 5.91            |       |                                      |                  |                 |
| H <sub>2</sub> O-                    | 3.15  | 2.00                   | 2.65             | 2.26            |       |                                      |                  |                 |
|                                      | 98.61 | 97.99                  | 99.23            | 98.63           | 100.0 | 100.0                                | 100.0            | 100.0           |
|                                      |       |                        | Norms            | Q               | 38.5  | 42.8                                 | 41.4             | 39.1            |
|                                      |       |                        |                  | Ab              | 13.5  | 15.0                                 | 15.0             | 15.0            |
|                                      |       |                        |                  | Ah              | 10.0  | 13.0                                 | 15.0             | 16.0            |
|                                      |       |                        |                  | An              | 30.0  | 8.5                                  | 8.0              | 9.5             |
|                                      |       |                        |                  | C               | 0.3   | 7.5                                  | 7.5              | 6.6             |
|                                      |       |                        |                  | En              | 5.8   | 6.2                                  | 6.2              | 6.2             |
|                                      |       |                        |                  | Il              | 1.4   | 1.4                                  | 1.4              | 1.4             |
|                                      |       |                        |                  | He              | 0.5   | 5.6                                  | 5.5              | 6.2             |

X-ray fluorescence: SiO<sub>2</sub>, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, MnO, CaO, K<sub>2</sub>O.

Flame photometry: Na<sub>2</sub>O.

Atomic absorption: MgO.



the medium fraction (2.96–2.6), and quartz and montmorillonite in the light fraction (<2.6). These findings give scant support for a volcanic origin of the material, although the amphibole crystals can hardly be considered to be secondary minerals and are therefore probably of igneous origin.

Four chemical analyses have been carried out on the three specimens; the first, which was unlabelled, is called X and the other two are called 5100–30 and 5220–50. A small sample, with a darker, slightly brownish tint, was hand picked from the 5220–50 which was predominantly light grey in colour (Table 1). The chemical differences between the four analysed materials were rather small. The large amount of normative corundum clearly indicates a strong secondary alteration of the glass with the formation of montmorillonite. We suspect that at least alkalis have been leached from the glass which has thus been subjected to the first stages of bentonitization. The primary composition of the ash may have been that of a quartz trachyte or rhyodacite, as indicated by the figures after omission of water and recalculation to 100%.

My findings suggest the existence of a region of explosive volcanism of Miocene age in the North Sea area. At present the well known areas of Tertiary volcanism around the North Sea are those of the Eocene basalts in western Scotland and the Faeroe Islands. Basaltic tuff beds also appear in the lower Eocene clays of Jutland, and the thickness of these increases towards the north. As early as 1903 Böggild wrote about the "Skagerrak volcanoes" which seem even more probable after a recent investigation by Madirazza and Fegerslev<sup>1</sup>. Sharma<sup>2</sup> verifies the existence of a "Kristiansand volcano" from the impressive anomaly on the aero-magnetic map, south of Kristiansand in the Norwegian Trench. This Kristiansand volcano may well be connected with the faulting of the Norwegian Trench<sup>3</sup>, the movements along which should then have started in the Eocene. Because no younger rhyolitic ash beds have been discovered in Jutland, the enormous Miocene explosions which gave rise to the central North Sea ash can hardly have originated at Kristiansand. It would be reasonable to place the Miocene explosive centre rather centrally in the North Sea, or possibly on the Norwegian Trench south-west or west of Stavanger. In the latter case the explosions could also date the faulting on the Norwegian Trench or the latter part of it. The explosions and the accompanying faulting may have occurred in association with the tilting and elevation of the west side of the Fennoscandian plate. The preliminary conclusion about the origin of the tuff formation is that it was formed during several air fall ash periods with short quiet periods in between.

The refractometer study was carried out by Mr J. Hysingjord, and the analysis by I. Rømme of this institute.

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## Deep Sea Photographs of an Active Seismic Fault Zone near Gibraltar Straits

IN October 1969, the RV Jean Charcot made a geophysical survey of the Horseshoe seamount and abyssal plain west of Gibraltar Straits and south-west of Cape Saint-Vincent, Portugal; this is the eastern end of the Azores–Gibraltar

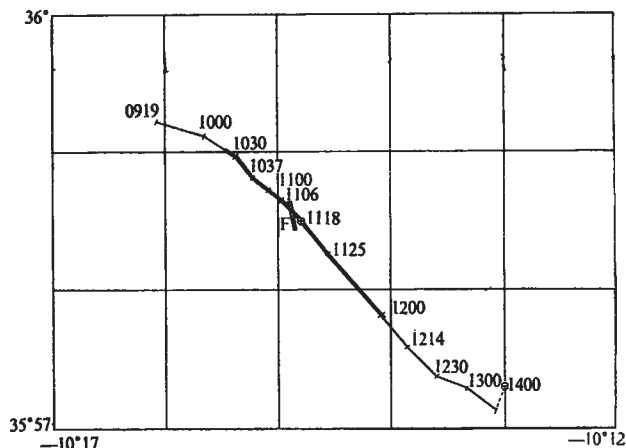


Fig. 1 The track of the troika (heavy line). F is the fault.

seismic zone<sup>1</sup> which is a compressive plate boundary<sup>2,3</sup>. During this survey a series of deep-sea photographs were obtained near the location of the last major earthquake of February 28, 1969 (magnitude 7.3 to 8.0, location 36.0° N 10.6° W according to the USCGS preliminary report).

The photographs were taken as a troika<sup>4</sup> was dragged along 3 km of seafloor (depth 4,800 m) in the south-eastern region of this turbidite abyssal plain which shows strong evidence of active tectonic deformation<sup>5</sup>. In all, 450 photographs (one every 12 s) were taken and the troika moved over the seafloor at an average speed of 2 km h<sup>-1</sup>. Navigation was performed by dead reckoning between satellite fixes, using electromagnetic lock and continuous course recording. The absolute accuracy is about 500 m and the relative accuracy is better than this. The position of the troika with respect to the ship was obtained from the difference in arrival time of direct and seafloor reflected acoustic waves from a 12 kHz pinger suspended 50 m above the troika. The absolute position of the troika is thus known with an accuracy better than 800–1,000 m (Fig. 1). The acoustic waves from the pinger also penetrated the mud to an average depth of 50–100 m and gave a reflexion profile below the troika (Fig. 2).

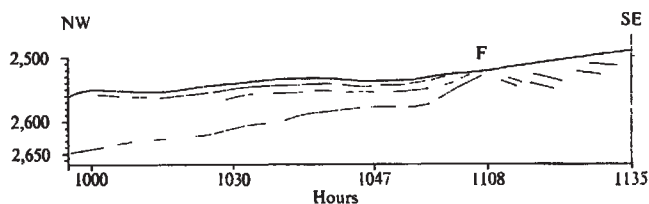


Fig. 2 Restituted seismic profile along the troika track from the 12 kHz pinger. F is the fault.

The path of the troika ran from north-west to south-east and was obliquely incident on the slope of a 200 m hill protruding above the floor of the abyssal plain. In the north-western part (west of 10° 15' W), the seafloor is flat and is typical of a turbidite abyssal plain; there is considerable evidence of animal life (burrowing and so on). Sediments seem to be made of ooze and silt; this is confirmed by a study of cores obtained in this plain which indicate a very high turbidite sedimentation rate (Needham and Pastouret, personal communications). A high sedimentation rate is also indicated by the presence of umbellulas (Laubier, personal communication). After 1050 h on Figs. 1 and 2 the floor begins to warp and the effect becomes progressively greater until 1106 h; at this time, the troika begins to climb a steep escarpment a few metres high. The photograph at 1108 h shows a fault plane oblique to the direction of the troika (Fig. 3). The vertical offset is about 120 cm and the visible length is about

8 m; the direction is slightly west of north. Gliding traces perpendicular to the fault can be seen on the up-side, which suggests that part of the recent sediment cover has slumped down the fault. The freshness of the gliding traces in this area of fast sedimentation (about 10 cm per thousand years) and the absence of signs of burrowing indicate that the movement along the fault has been very recent.

Fig. 4 shows a close-up of the fault plane and many holes can be seen. These are the only examples of such holes in the whole series of photographs. (G. P. has seen similar holes, with bubbles of gas coming out of them, during a bathyscaphe dive along the Provence continental slope. He suggests that they are caused by degassing of the sediment.) The fact that these holes seem unperturbed is further evidence of very recent movement. From 1108 h to 1200 h the sea bottom is warped to the extent of a few metres (Fig. 5) and has a relatively smooth surface. The succession of photographs suggests that the track followed by the troika was nearly parallel to a major escarpment on top of which is the fault plane photographed at 1108 h. The escarpment was apparently created by normal faulting accompanied by large scale slumping.

A flexotir profile was obtained across the edge of the hill. The hill corresponds to an uplift of a zone opaque to acoustic waves and on each side the upper layer of post-Miocene turbidites<sup>5</sup> is upbent. The opaque zone consists of semiconsolidated sediments with a seismic velocity of 3–4 km s<sup>-1</sup> which have been heavily tectonized<sup>5</sup>. This confirms our interpretation. The hill is formed by the uplift of this mass of tectonized sediments which is accompanied by normal faults along the edges, upbending of the adjacent turbidites and some slumping.

There seems to be good agreement between the conclusions drawn from deep sea photographs, bathymetry, seismic profiling of the upper cover from the seafloor and flexotir profiling from the sea surface. A detailed mapping of the present

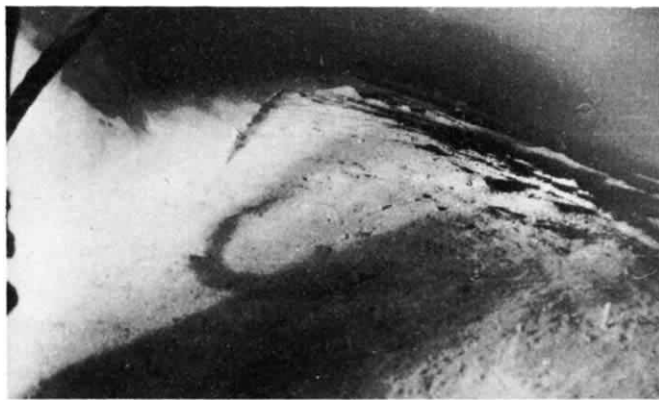


Fig. 5 Photograph taken at 1120 h showing the large scale warping of the seafloor over the slope of the hill.

tectonics of the upper sedimentary cover could be undertaken using this type of systematic surveying.

It has, however, been shown that the fault plane mechanisms along this plate boundary are thrust faulting toward the north<sup>3,6</sup>. Le Pichon *et al.*<sup>5</sup> have confirmed that this abyssal plain can be interpreted as an incipient trench. This apparent contradiction between deep crustal tectonics and superficial tectonics can be explained by the difference in mechanical properties of the crust and sedimentary cover. As the basement is being swallowed along the zone on thrusting at the bottom of the abyssal plain, the incompetent and lighter sedimentary cover overflows on each side of the plain. Deep sea studies of the microseismicity may also give some indications on the tectonics of the sedimentary cover and such a study is in progress.

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## Probable Non-synchronicity of Late Precambrian Glaciations

THE palaeomagnetic confirmation<sup>1,2</sup> of King's suggestion<sup>3</sup> that the area affected by Upper Palaeozoic glaciation in Gondwanaland changed its position with time is important in itself, and geological arguments substantiating this change have been given in detail<sup>4</sup>. A further impressive relationship has been established between a pole position for the Ordovician<sup>5</sup> and previously discovered evidence of a continental glaciation in North-West Africa during the Late Ordovician and Early Silurian<sup>6–8</sup>. Thus the Palaeozoic polar wander curve for the African continent<sup>5</sup> is in good agreement with



Fig. 3 Photograph of the fault taken at 1108 h.



Fig. 4 Close-up of the fault plane and the holes due to degassing of the sediment.



the geological evidence for progressive shifts in the centres of glaciation (presumably polar) during the Palaeozoic.

These discoveries are highly relevant to the age of Late Precambrian glaciations, for which there is abundant evidence in both northern and southern hemispheres. In the southern hemisphere, good examples of very thick sequences of tillites and associated glaciogene rocks occur in South Australia<sup>9</sup>, which show recurrence of glaciation and imply at least one important interglacial. Similar examples occur in western New South Wales<sup>10</sup>, in central Australia<sup>11</sup> and in the Kimberley district of Western Australia where tillites frequently overlie glaciated pavements<sup>12,13</sup>. Comparable evidence exists in the Congo<sup>14</sup>, Angola<sup>15</sup>, Zambia<sup>16</sup>, South and South-West Africa<sup>17-19</sup> and South America<sup>20</sup>. In the northern hemisphere there is evidence in the United States<sup>21</sup>, Canada<sup>22</sup>, Greenland<sup>23</sup>, Spitzbergen<sup>24</sup>, Scandinavia<sup>25</sup>, the British Isles<sup>26,27</sup>, Normandy<sup>28</sup>, Czechoslovakia<sup>29</sup>, West Africa<sup>30-32</sup>, central China<sup>33-35</sup>, Chinese Turkestan<sup>36</sup>, and in the USSR in Belorussia, the Urals, Khazakhstan, the Tien-Shan, central and far north-east Siberia<sup>37</sup>. Evidence from India, however, is poor<sup>38</sup>.

Most of these occurrences are well established but some are occasionally disputed<sup>39</sup>. All of them occur in rock sequences in the age range between 1,000 m.y. and the base of the Cambrian. Other evidence of Precambrian glaciation comes from substantially older rocks in both the northern and southern hemispheres.

It has commonly been believed by stratigraphers working on the Precambrian, particularly in the southern hemisphere, that tillites, which are widespread, would provide useful time-stratigraphic markers in the Late Precambrian. Harland<sup>40</sup> suggested that they may be the best basis for defining internationally recognizable Precambrian systems. Harland suggested that the Late Precambrian glaciation had thermal and eustatic effects which controlled animal life, evidently believing that it was synchronous throughout the world. He and Rudwick<sup>41</sup> proposed glaciation as a trigger control to account for the widespread appearance of fossils in the Lower Cambrian.

The worldwide synchronicity of Late Precambrian glaciation is not likely. Ice-sheet glaciation is a polar feature and the area which it affects moves with the poles. Glaciation is not a necessary feature, but if it does occur, it is essentially circumpolar, however variable its intensity and its radial extent towards the equator of the time. This is quite independent of continental drift which may later scatter the glaciation evidence of any one area.

No Precambrian glaciation has so far been accurately dated. In Australia, the age of the Sturt Tillite is still uncertain within wide limits<sup>42</sup>. Bofinger<sup>43</sup> reports an age of 740 m.y., with an internal precision of  $\pm 30$  m.y., for the glacial shales of the east Kimberley region. There is an additional uncertainty because he uses a particular interpretation of the Rb-Sr dating of shales. No other dating of Precambrian glacials in Australia has been attempted. There is an uncertainty in the age of glaciation for almost every other example, though Keller *et al.*<sup>44</sup> quote a time span from 675 to 560 m.y. for the Vendian of the USSR, and suggest that an inland ice-sheet affected northern Europe during this time. We think that when accurate data become available, areas of glaciation will be found to lie along the polar wander curve of the Late Precambrian, and will show a similar shift to that of the Late Palaeozoic glaciation. McElhinny *et al.*<sup>5</sup> have already demonstrated polar wandering for the Precambrian of southern Africa but the data presented do not include the Late Precambrian between 1,300 m.y. and the base of the Cambrian. The use of Precambrian tillites as time-stratigraphic markers with the implication of synchronicity is, therefore, almost certainly incorrect. Consequently, tillites should be quite unsuitable for delimitation of Precambrian systems. It is also unlikely that worldwide biological changes can be related in time to the effects of glaciation. Instead, we should be able

to show that glaciation has itself been a recurrent feature, theoretically possible at any time in polar regions, but for reasons doubtless connected with the Sun, the atmosphere and the oceans, not invariably present. It seems to us that these ideas should be tested by continuing efforts to date the various glacial deposits by isotopic and palaeontological means.

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## BIOLOGICAL SCIENCES

## Effect of Lithium on Human Aggression

LITHIUM has been shown to be effective in the manic and hypomanic phase of manic-depressive psychosis<sup>1-3</sup>, and to some extent to prevent manic episodes and, to a lesser degree, depressive episodes<sup>4-6</sup>. Manic depressive disorders are frequently characterized by hyperaggressive and hypersexual activity. In animals lithium has been reported to have an inhibitory effect on aggressive behaviour<sup>7,8</sup> and on the sexual and aggressive behaviour seen with p-chlorophenylalanine<sup>9</sup>. I therefore conducted a clinical trial of lithium on human aggressive behaviour.

The subjects used in this study were twelve male inmates of a maximum-security state prison at Somers, Connecticut, age range 21-43, mean 31; nine Caucasian and three Negro. They were selected on the basis of (1) pre-prison history of three or more episodes of violent assaultive crime; (2) prison behaviour characterized by continuing verbal and physical aggressive behaviour; (3) no overt psychosis or brain damage; (4) no renal or cardiovascular diseases; (5) high scores on aggression items of MMPI and Buss rating scale for aggression; and (6) IQ over 85 on group Stanford-Binet test.

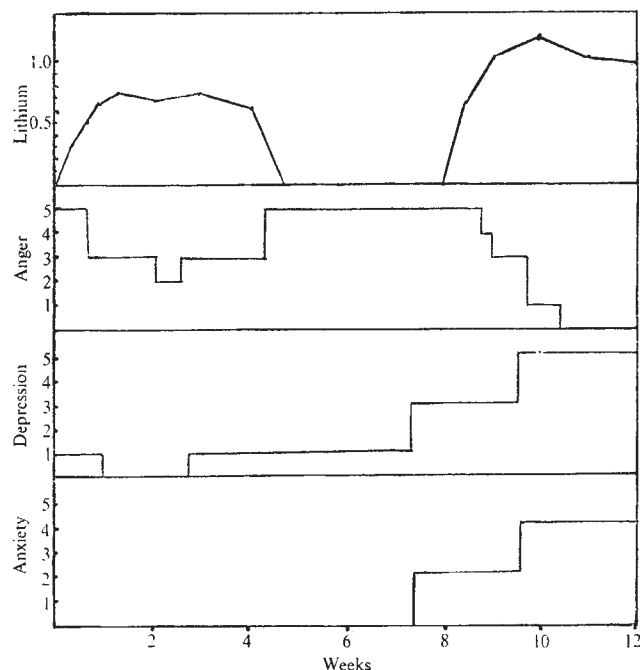
All subjects had a routine physical check-up, including complete blood counts, urinalysis, chest X-ray, electrocardiograph, serum glutamate transaminase, and protein bound iodine. Somatotyping was performed using the method of Parnell<sup>10</sup>, a method of measuring body type with three components (fat-endomorphy, muscularity-mesomorphy and linearity-ectomorphy) each scored on a scale of 1-7.

An assessment of changes was made on the basis of the following criteria. A self rating scale with ten items rated (1-5) for intensity daily each evening. Items were: feeling hopeless, worthless, helpless, ashamed or guilty, nervous or afraid, angry, sad, suspicious, tense, and difficulty sleeping. A symptom check list was filled in weekly, and the number of incidents of verbal or physical aggressiveness serious enough to merit "tickets" received from administrative staff for violation of rules were counted. Hostility in verbal behaviour was rated during a clinical interview of 15 min, once weekly. Serum lithium was measured by atomic absorption spectrophotometry. This initial pilot study was a single blind trial with drug and placebo capsules of identical appearance and taste. Placebo and drug were given for 4 week periods in an alternating sequence. Subjects were randomly allotted to placebo or drug first. Total length of trial was 3 months. Lithium was administered in the form of the carbonate in a 300 mg capsule (Rowell Laboratories). Weekly blood samples were taken and serum lithium maintained between 0.6 meq. and 1.5 meq./l. by adjustment of daily dose. The average dose required was 1,200 mg daily.

**Table 1** "Mean Aggressive Affect Rating" and "Mean Number of Reports for Aggressive Behaviour" under Lithium or Placebo Treatment

| Treatment | No. | Mean aggressive affect rating (0-5) | Mean No. reports for aggressive behaviour per month |
|-----------|-----|-------------------------------------|-----------------------------------------------------|
| Placebo   | 12  | $3.5 \pm 0.75$                      | $3.8 \pm 0.75$                                      |
| Lithium   | 12  | $1.5 \pm 0.5$ $P < 0.01$            | $0.75 \pm 0.25$ $P < 0.01$                          |

There was a significant reduction in aggressive affect (self rate items of anger and tension) associated with a reduction in number of tickets received for physical or verbal aggression in lithium periods contrasted with placebo periods (Table 1;  $P < 0.01$ ). In the dose range used in the study, side effects were uncommon. Most common were complaints of mild nausea and loss of appetite for the first 2 or 3 days (five cases),



**Fig. 1** Individual somatotype 1.5, 2, 5. Changes in mood levels of anger, depression and anxiety associated with serum levels of lithium (meq./l.) over a 12 week period. Placebo 4-8 weeks.

an increase in thirst (four cases); and sleeplessness was more frequently recorded (eight cases). A mild tremor was observed in two cases. An analysis of the somatotype data showed that there was a tendency for the twelve individuals to fall into two groups: a mesomorphic group (seven cases—wide heavy—typical somatotype 4, 5, 2) and an ectomorphic group (five cases—tall thin—typical somatotype 3, 2, 6). There was a tendency for mesomorphs to show a better overall response to lithium than the more ectomorphic types. Overall response was a global assessment based on a reduction of two points or more in scores of self rated items of anger, tension and tickets for aggressive behaviour without an increase of more than one point in self rated items of depression (combined scores on items of worthlessness, helplessness and sadness) as anxiety (self rated items of fear or nervousness). In three individuals whose aggressive behaviour could be said to be an integral aspect of personality style a reduction in aggressive affect was associated with a concomitant increase in anxious and depressive affect. A typical example is shown in Fig. 1 (somatotype 1.5, 2, 5). But for four individuals in whom the tendency to act destructively was a source of anxiety, the result of lithium was to reduce aggressive affect and tension without an increase in depressive or anxiety affect. A typical example of this type is seen in Fig. 2 (somatotype 4, 5.5, 2.5). The results support the conclusion that lithium can be useful in the treatment of selected instances of human aggression. It should be noted that this study was not double blind and hence is subject to observer bias. Two of the assessment tools were made under blind conditions, however—the self-rating scales and the number of tickets given by prison staff for aggressive behaviour. Both of these showed significant differences between drug and placebo condition ( $P < 0.01$ ).

In view of the report of sleeplessness (placebo score  $2.5 \pm 0.5$ , lithium score  $4 \pm 0.5$ ,  $P < 0.01$ ) it is interesting that aggression was diminished in spite of the tendency to increased irritability and aggressiveness associated with loss of sleep.

That this type of constitution responds well to lithium is interesting in view of the poor reaction to drugs in general reported by Klerman *et al.*<sup>11</sup> for the mesomorphic constitution. Whether inhibition of aggression by lithium has favourable or unfavourable results seems to depend on the adaptive



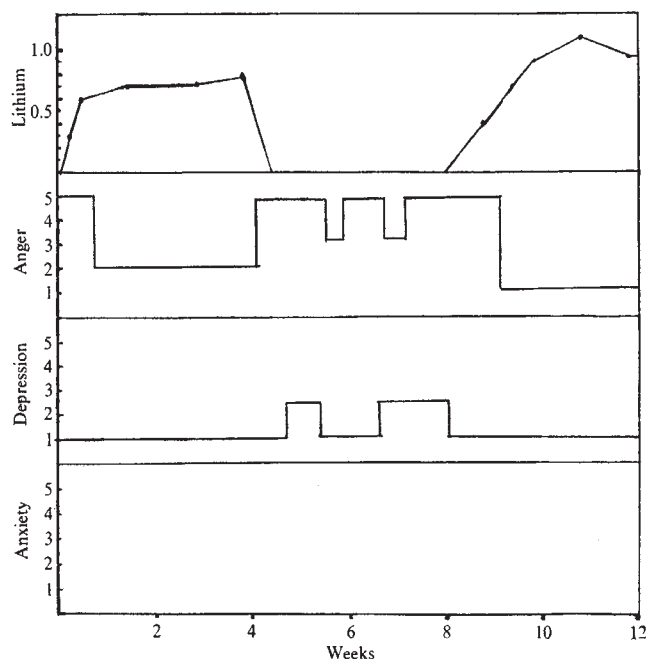


Fig. 2 Individual somatotype 4, 5.5, 2.5. Changes in mood levels of anger, depression and anxiety associated with serum levels of lithium (meq/l.) over a 12 week period. Placebo 4-8 weeks.

significance of the aggressive behaviour for the individual, what opportunities exist for the development of other behaviour patterns, and constitutional factors.

The mechanism of action of lithium is not known. It is known, however, that lithium can enhance the metabolism of serotonin<sup>12</sup>, and lowering of serotonin has been shown to enhance the release of aggressive behaviour in animals' brains<sup>13</sup>.

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## Effects of Maternal Preimmunization on the Decidual Cell Reaction in Mice

ALTHOUGH several hypotheses have been put forward to explain the success of the mammalian conceptus as a homo-graft<sup>1</sup>, there is still no agreement on the exact mechanisms involved. It has been postulated that during the early stages of pregnancy the uterine decidual tissue prevents the immunological consequences of antigenic differences between mother and conceptus<sup>2</sup>. Recent evidence has suggested that the extent of the decidual cell reaction is reduced when there are genetic differences between mother and offspring<sup>3</sup>. We have therefore investigated whether the decidual cell reaction is affected by preimmunization of the mother to paternal antigens.

CBA/Fa and C57BL/Fa inbred mice which differ at the  $H_2$  locus were used. C57BL female mice aged between 8 and 12 weeks were distributed at random into three groups. The first group was immunized allogeneically to CBA male mice, the second group was immunized to cells from *Peromyscus maniculatus bairdii* and *Peromyscus polionotus polionotus* (deer mice), to test the effects of xenogeneic immunization, and the third group was left untreated, as controls. The immunization procedure in both instances consisted of two full thickness skin grafts<sup>4</sup>, followed by three spleen cell injections (0.5 spleen equivalent each). All treatments were given at 14 day intervals. Skin grafts of both *Peromyscus* and CBA healed satisfactorily, and second set rejection times were  $6 \pm 2$  days for both the allogeneically and xenogeneically immunized groups. Six days after the last injection all mice were mated to CBA males. On the seventh day of pregnancy (the day of vaginal plug representing the first day) mice were killed, and serum was collected from each female for titration of antibody. Decidual tissue was dissected from the uterus, in physiological saline; no attempt was made to remove the tissue of the conceptus. The decidua from each uterus were blotted lightly, pooled and weighed to the nearest 0.1 mg. The mean decidual weight per implantation site was calculated for each female.

Data were subjected to an analysis of variance. The mean decidual weight from females immunized to CBA antigens was significantly less than the mean weight of either the untreated control group or the group immunized to *Peromyscus* ( $P < 0.001$ , Table 1). There was no significant difference between the untreated controls and the xenogeneically immunized females. The effect of immunization with cells of the paternal strain in decreasing decidual weight is therefore not a result of a general heightening of a non-specific immune reaction nor of stress experienced during immunization.

Maternal antibody to red cells was measured by the serum-dextran method of Gorer and Mikulska<sup>5</sup>. Sera from allo-

Table 1 Decidual Weights, Lumbar Node Weights and Spleen Weights in Allogeneically Immunized, Xenogeneically Immunized and Non-immunized Female Mice

|                         | No. of females | Mean implant number $\pm$ s.e. | Mean decidual weight (mg) $\pm$ s.e. | Lumbar nodes weight (mg) $\pm$ s.e. | Spleen weight (mg) $\pm$ s.e. |
|-------------------------|----------------|--------------------------------|--------------------------------------|-------------------------------------|-------------------------------|
| Allogeneic immunization | 14             | 7.5 $\pm$ 0.6                  | 2.29 $\pm$ 0.13                      | 6.31 $\pm$ 0.4                      | 122.1 $\pm$ 4.1               |
| Xenogeneic immunization | 17             | 8.2 $\pm$ 0.4                  | 3.25 $\pm$ 0.20                      | 7.31 $\pm$ 0.6                      | 126.2 $\pm$ 4.2               |
| Controls                | 17             | 8.6 $\pm$ 0.3                  | 3.27 $\pm$ 0.15                      | 3.97 $\pm$ 0.4                      | 119.9 $\pm$ 2.9               |

genically immunized females produced haemagglutination titres ranging from 1/8 to 1/4,096. Sera from xenogeneically immunized mice gave high haemagglutination titres (1/1,024 to 1/32,768) when tested against *Peromyscus* red blood cells, but showed no cross reactivity when tested against CBA antigens. The control females showed no activity with either antigen. Lumbar lymph nodes and spleens were dissected from each female at autopsy and weighed. Immunized mice (both xenogeneic and allogeneic) had significantly larger lymph nodes ( $P < 0.001$ ) and spleens ( $P < 0.05$ ) than controls. The mean implantation number in the allogeneically immunized mice was lower than in the other groups but the reduction was not significant (Table 1).

There are several possible explanations for the effect of immunization to paternal antigens. The time of implantation of the embryo may be delayed, the decidual stimulus produced by the blastocyst may be reduced or the mother's ability to respond to the inducing stimulus may be impaired. Alternatively, specific immunization may directly decrease the rate of growth of the decidual tissue. It is not possible at present to distinguish between these alternatives.

In previous experiments involving maternal immunization to paternal antigens Mitchison<sup>6</sup> found no adverse effects on the outcome of a pregnancy. James<sup>7</sup>, however, claimed that maternal sensitization to paternal antigens increased placental and foetal weight. Clarke<sup>8</sup> was unable to confirm these results and found that when C57BL/Fa females were immunized to CBA/Fa antigens, placental weight was unaffected although foetal weight was reduced. This reduction, in conjunction with the reduction in decidual weight observed in this experiment, is in agreement with the observations of Hetherington<sup>3</sup>, who found a positive association between decidual weight on the seventh day of pregnancy and foetal weight on the eighteenth day. It is not known whether the reduction in foetal weight is the direct consequence of the reduced decidual response or whether both foetal and decidual weights are dependent on a third factor.

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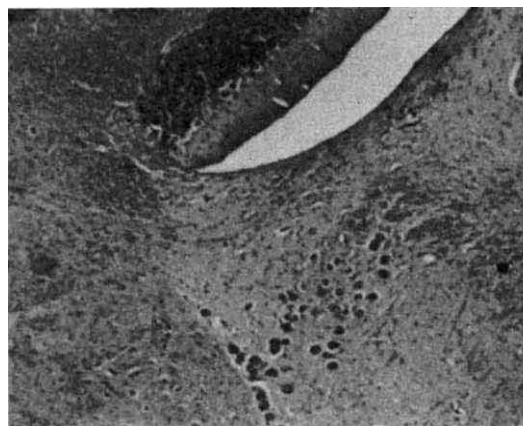
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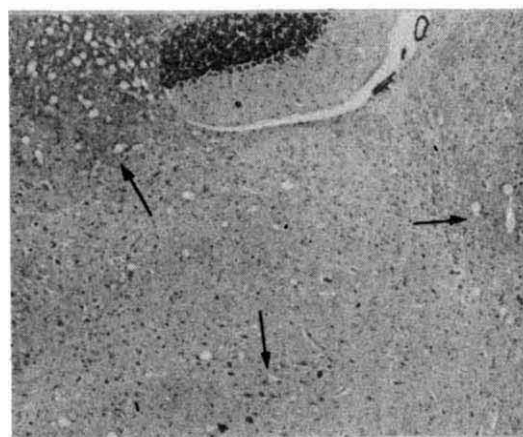
## Histopathological Similarities between Scrapie and Cuprizone Toxicity in Mice

PREVIOUS work with scrapie led us to conclude<sup>1</sup> that the transmissible agent was unlikely to be a conventional virus. We have presented evidence<sup>2</sup> that the transmissible agent may be, or may be associated with, a small basic protein (polypeptide) of the kind involved in the production of experimental allergic encephalomyelitis, and we have recorded the possible occur-

rence of the agent in tissues of normal animals<sup>3-5</sup>. These observations have neither been confirmed nor disproved in other laboratories, and various other hypotheses on the nature of scrapie and its transmissible agent remain unproved<sup>6-8</sup>. The "slow virus" hypothesis still favoured by some<sup>9</sup> is now at variance with the impressive experimental evidence from ultraviolet irradiation studies<sup>10-13</sup> that the transmissible agent may not contain nucleic acid.



a



b



c

Fig. 1 Extracellular vacuolation in the cerebellum, pons and mid-brain areas (arrows) of brains of scrapie-affected mice or mice fed cuprizone. a, Normal; b, scrapie; c, cuprizone. (Haematoxylin and eosin,  $\times c. 40$ .)



Our attention was drawn some months ago to articles by Carlton<sup>14-16</sup> describing spongiform encephalopathy produced in mice, rats and guinea-pigs by the addition of cuprizone (biscyclohexanone oxaldihydrazone) to the diet. We were impressed by the similarity of the brain lesions described and depicted in these publications to those of scrapie.

The basic abnormality in all species was brain oedema characterized by vacuole formation which was most extensive in the white matter of the cerebellum and mid-brain. Of the

three species studied, the mouse was most susceptible to cuprizone toxicity, and in the mouse and rat "large naked glial nuclei resembling Alzheimer's type II glia" were conspicuous. Small focal areas of myelin loss were observed in mice within and adjacent to the spongy areas, and hydrocephalus was common in younger animals.

We have compared this disease with scrapie in mice by carrying out three experiments in which groups of 11, 11 and 67 mice respectively, of the BSVS strain bred at Compton and 4-6 weeks old, were fed exclusively on a powdered meal diet containing 0.5% by weight of cuprizone (Koch-Light), with water freely available. As Carlton noted, this level of cuprizone was quite toxic, and during the 60-day experimental period 18 of the 89 mice were found dead and were unsuitable for examination. Brains of the remaining 71 were examined histologically at times ranging from 5 to 59 days after starting cuprizone feeding. The stains used on formalin-fixed tissue were haematoxylin and eosin, and Cajal's gold chloride sublimate for astrocytes.

The principal histological findings were as described by Carlton, and their similarity to those of scrapie is shown in Figs. 1 and 2. In particular, attention is drawn to the location of the extracellular vacuolation in the cerebellar white matter, the pons and mid-brain areas (Fig. 1), and to the hypertrophy of astrocytes (Fig. 2). Although astrocytes were reactive quite early in the cuprizone encephalopathy, the hypertrophy that is so characteristic of the astrocyte in scrapie was not obvious until about the 35th day of cuprizone feeding. Carlton did not comment on the strict bilateral symmetry of the lesions in cuprizone encephalopathy, but this was clearly visible in transverse sections of brain and is, of course, an outstanding feature of the pathology of scrapie.

Carlton found that copper supplementation of a diet containing a toxic amount of cuprizone did not prevent development of oedema and spongy degeneration, and he concluded that cuprizone may have a direct toxic effect that is not mediated by copper chelation. He suggested that this toxicity might be one of enzyme inhibition, possibly of amine oxidase.

Spongiform encephalopathy is a common link between the four slowly progressive degenerative diseases of the central nervous system that have been transmitted experimentally to animals, namely scrapie, kuru, Creutzfeldt-Jakob disease and mink encephalopathy. It therefore seems to us that the study in greater depth of the spongiform encephalopathy produced by cuprizone might lead to better understanding of these diseases.

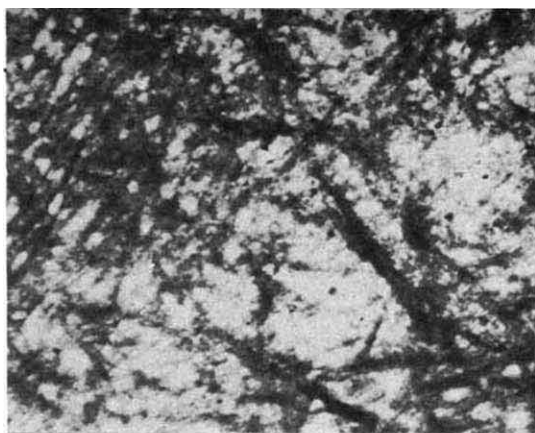
We thank Mr P. F. Dennis for histological preparations, Mr I. M. H. Jebbett for photographs, and Mr F. W. C. Howard and Miss J. M. Mundy for care of the animals.

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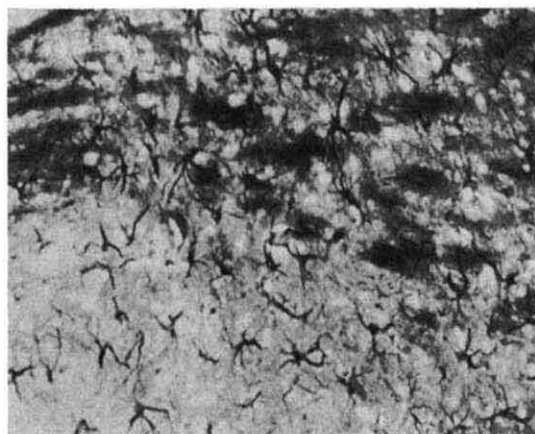
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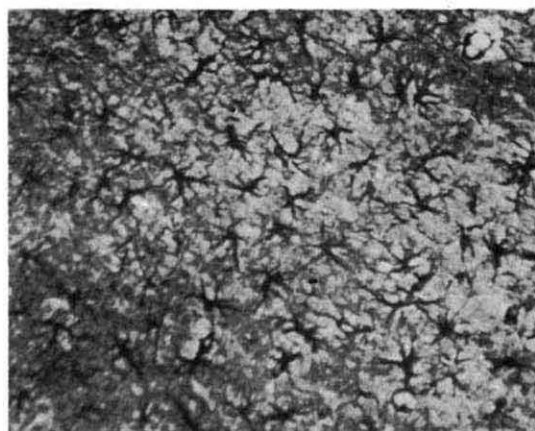
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a



b



c

Fig. 2 Astrocyte hypertrophy in the mid-brain area of scrapie-affected mice or mice fed cuprizone. a, Normal; b, scrapie; c, cuprizone. (Cajal,  $\times c. 40$ .)

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## Antiviral Effect of Constituent Parts of the Rifampicin Molecule

THE antibiotic rifampicin inhibits the growth of bacteria by inhibiting DNA dependent RNA polymerase<sup>1,2</sup>. It also inhibits the growth of vaccinia and other poxviruses<sup>3,4</sup>. An interaction between the antibiotic and the viral polymerase has not been demonstrated, however, and the antiviral mode of action of the drug remains unknown.

The rifampicin molecule is a condensation product of 3-formyl rifamycin SV and 1-amino 4-methyl piperazine (Fig. 1). It has recently been claimed<sup>5</sup> that 1-amino 4-methyl piperazine has antiviral properties similar to those of rifampicin. We have been unable to confirm this finding either with 1-amino 4-methyl piperazine or with the hydrochloride, and here we present evidence to suggest that 1-amino 4-methyl piperazine has no specific antiviral effect and that the antiviral activity of rifampicin resides in the rifamycin part of the molecule.

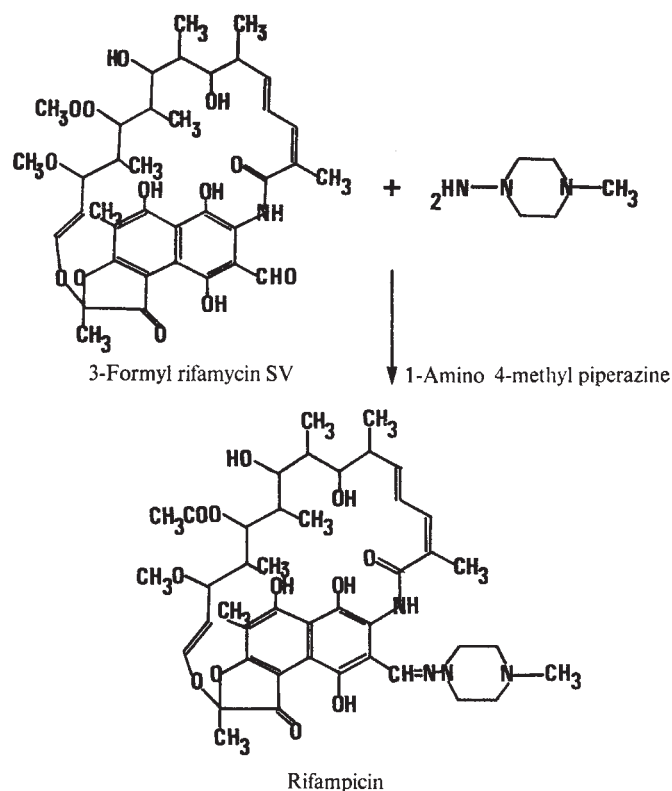


Fig. 1

Plaque assays of vaccinia virus (Evans vaccine strain) in BHK 21/C13 monolayers in the presence and absence of 1-amino 4-methyl piperazine at concentrations up to 500 µg/ml. in a fluid overlay gave no evidence of any significant antiviral effect. Even at 1 mg/ml. the effect, if any, was small. One such experiment is shown in Table 1. In identical conditions rifampicin (100 µg/ml.) completely inhibited plaque formation. When incorporated in an agar overlay, 1-amino 4-methyl piperazine at a concentration of 500 µg/ml. did not significantly inhibit virus plaque formation, and at 1 mg/ml. the drug had a severe cytotoxic effect which made observations on plaque formation impossible.

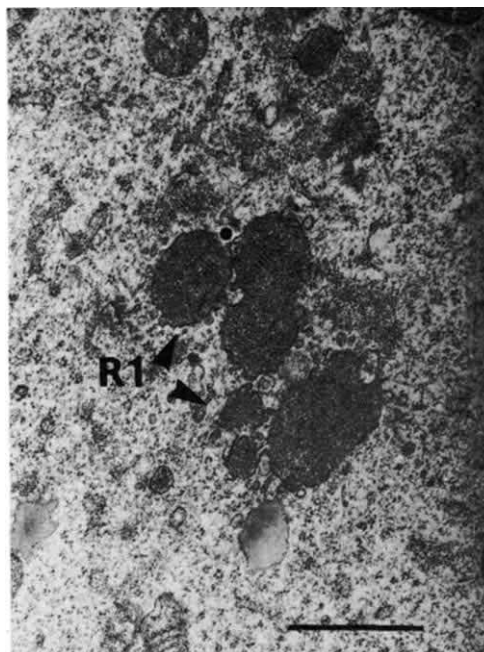


Fig. 2 Thin section of C13 cell maintained in 100 µg/ml. rifampicin for 17 h. Only electron-dense R1 inclusions can be seen. Bar = 1 µm.

The antiviral effect of rifampicin is reversible<sup>6-9</sup>; removal of the drug late in the virus growth cycle results in the production of mature infectious virus within 1 h. We therefore examined the effect of 1-amino 4-methyl piperazine on the events which follow the removal of rifampicin, reasoning that if 1-amino 4-methyl piperazine acts in a similar way to rifampicin the replacement of rifampicin by 1-amino 4-methyl

**Table 1** Vaccinia Virus Plaque Counts in C13 Cells in the Presence or Absence of 1-Amino 4-Methyl Piperazine contained in Liquid or Agar Overlay

| Concentration of 1-amino 4-methyl piperazine (µg/ml.) * | Plaque counts  |     |              |    |
|---------------------------------------------------------|----------------|-----|--------------|----|
|                                                         | Liquid overlay |     | Agar overlay |    |
| 0                                                       | 359            | 419 | 53           | 51 |
| 500                                                     | 410            | 311 | 42           | 41 |
| 1,000                                                   | 203            | 145 | —            | —† |
| Rifampicin 100 µg/ml.                                   | 0              | 0   | 0            | 0  |

\* Medium containing 1-amino 4-methyl piperazine was added after a 1 h adsorption period. Plaques were counted after 48 h incubation at 37° C.

† Pronounced cytotoxic effect.

**Table 2** Effect of 1-Amino 4-Methyl Piperazine on Virus Growth after the Removal of Rifampicin

| Drug added after removal of rifampicin | Time of incubation after removal of rifampicin (h) | Titre (plaque forming units/plate) |
|----------------------------------------|----------------------------------------------------|------------------------------------|
| None                                   | 0                                                  | $6.8 \times 10^4$                  |
| None                                   | 4                                                  | $1.0 \times 10^6$                  |
| 1-Amino 4-methyl piperazine (1 mg/ml.) | 4                                                  | $6.8 \times 10^5$                  |
| Rifampicin (100 µg/ml.)                | 4                                                  | $7.0 \times 10^4$                  |

C13 monolayers were infected with an input multiplicity of 5 plaque forming units/cell and were incubated in medium containing rifampicin (100 µg/ml.). At 17 h after infection 1-amino 4-methyl piperazine was added to the plates indicated and incubation was continued for a further 10 min. The medium from all plates was then removed and replaced with fresh medium containing the drugs indicated above.



**Table 3** Electron Microscope Observations on Vaccinia Virus Maturation following the Removal of Rifampicin and Subsequent Addition of Various Components

| Drug added after removal of rifampicin                    | R1 inclusions | Immature particles developing at the periphery of R1 inclusions (IP) | Immature particles (I) | Immature particles with dense nucleoids (Id) | Mature virus particles (M) |
|-----------------------------------------------------------|---------------|----------------------------------------------------------------------|------------------------|----------------------------------------------|----------------------------|
| None                                                      | —             | +                                                                    | +                      | +                                            | +                          |
| 1-Amino 4-methyl piperazine                               | —             | +                                                                    | +                      | +                                            | +                          |
| Rifampicin                                                | +             | —                                                                    | —                      | —                                            | —                          |
| 3-Formyl rifamycin SV                                     | +             | —                                                                    | —                      | —                                            | —                          |
| 3-Formyl rifamycin SV 1.5 h followed by normal medium 4 h | —             | +                                                                    | +                      | +                                            | +                          |

Monolayers of C13 fibroblasts were infected at a multiplicity of 5 plaque forming units/cell. After incubation for 17 h in medium containing 100 µg/ml. rifampicin, the medium was replaced with medium containing 100 µg/ml. rifampicin and either 1 mg/ml. 1-amino 4-methyl piperazine or 3-formyl rifamycin SV (200 µg/ml.). After a further 30 min incubation this medium was exchanged for medium containing either only 1 mg/ml. 1-amino 4-methyl piperazine or only 3-formyl rifamycin SV (200 µg/ml.). In control cultures, medium containing rifampicin was replaced with normal medium or with medium containing rifampicin. After a further 2 h incubation the cells were fixed in glutaraldehyde and osmium tetroxide and embedded in epon as described previously<sup>10</sup>. Thin sections were picked up on uncoated grids and stained with uranyl acetate and lead hydroxide.

piperazine should prevent subsequent maturation of the virus. This we did not find. A substantial increase of infectivity occurred after replacement of rifampicin by 1 mg/ml. 1-amino 4-methyl piperazine (Table 2). We have extended these observations by following the morphological changes in infected cells before and after the removal of rifampicin and its replacement with 1-amino 4-methyl piperazine. In cells infected and subsequently maintained in the presence of rifampicin numerous electron-dense inclusion bodies (R1) accumulate<sup>8,9</sup> (Fig. 2). Neither immature nor mature particles are seen. After the removal of rifampicin immature particles (IP) form at the periphery of these inclusions<sup>8,9</sup> (Fig. 3). These particles subsequently develop dense nucleoids (Id, Fig. 3) and eventually mature virus particles appear (M, Fig. 3). When rifampicin was removed and replaced by 1-amino 4-methyl piperazine (1 mg/ml.) immature forms developed at the periphery of the R1 inclusions and subsequent stages in the

development of the mature virus particle proceeded normally (Fig. 3; Table 3). Our results therefore provide no evidence that 1-amino 4-methyl piperazine possesses antiviral properties similar to those of rifampicin.

Because prolonged exposure of C13 cells to 3-formyl rifamycin at high concentrations produces toxic effects, we have been unable to assess the antiviral properties of this compound by plaque assay. Infected cells survive short exposure to 3-formyl rifamycin, however. After preincubation of infected cells with rifampicin the drug was removed and replaced by 3-formyl rifamycin and electron microscopic observations made at intervals. When rifampicin is replaced by normal medium immature particles develop and some final maturation of the virus occurs within 1 h<sup>8,9</sup>. But replacement of rifampicin by 3-formyl rifamycin (200 µg/ml.) did not result in the formation of immature particles in any of our experiments (Table 3), R1 inclusion bodies remaining apparently unchanged. Subsequent removal of 3-formyl rifamycin allowed maturation of the virus. This suggests that in our system the antiviral activity of rifampicin resides in the rifamycin moiety of the molecule.

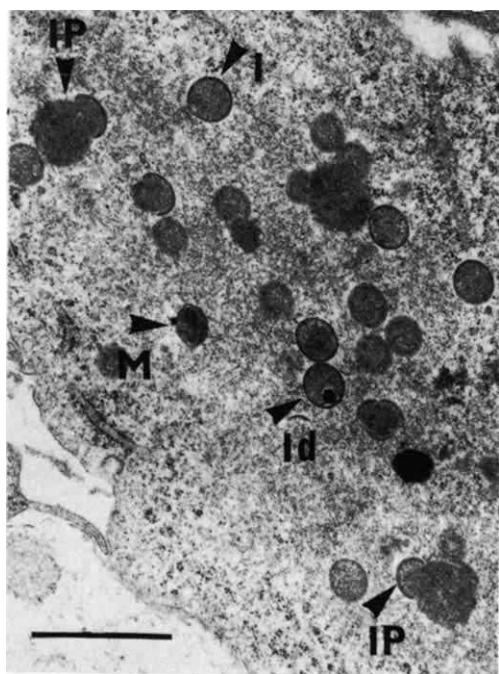
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**Fig. 3** Thin section of C13 cell maintained in 100 µg/ml. rifampicin for 17 h. The rifampicin was removed and replaced by 1 mg/ml. 1-amino 4-methyl piperazine for a total of 2.5 h. Immature particles forming at the periphery of electron-dense inclusions (IP), immature particles (I), immature particles with dense nucleoids (Id) and a mature particle (M) are now evident. Bar = 1 µm.

## Tissue Perfusion measured using the Ratio of $^{81}\text{Rb}$ to $^{81\text{m}}\text{Kr}$ incorporated in the Tissue

RUBIDIUM, when introduced intravenously, behaves similarly to potassium, being incorporated principally within the intracellular pool<sup>1,2</sup>. The radionuclide  $^{81}\text{Rb}$  (refs. 3-5), produced in a cyclotron, decays with a half life of 4 h 34 min to  $^{81\text{m}}\text{Kr}$ , which in turn decays with a half life of 13 s to stable  $^{81}\text{Kr}$ . Therefore the amount of  $^{81}\text{Rb}$  within a tissue after administration changes only slowly<sup>1</sup>, whereas its daughter, being inert, mixes uniformly with the tissue<sup>6</sup> and is removed from it at a rate dependent on the perfusion of the tissue. At equilibrium the ratio of  $^{81\text{m}}\text{Kr}$  to  $^{81}\text{Rb}$  within a tissue is given by:  $\lambda_1/(\lambda_2 + K)$ , where  $\lambda_1$  and  $\lambda_2$  are the disintegration rate constants of  $^{81}\text{Rb}$  and  $^{81\text{m}}\text{Kr}$  respectively and  $K$  the perfusion rate constant of the tissue. Thus the relationship between  $^{81}\text{Rb}$  and its inert gas daughter could provide a unique method for measuring tissue perfusion.

The error in such a measurement due to recirculation of  $^{81\text{m}}\text{Kr}$  is negligible, because of the 13 s half life of  $^{81\text{m}}\text{Kr}$  and its release into the alveoli from the lung capillary blood. The energies of the principal  $\gamma$ -rays emitted by  $^{81}\text{Rb}$  and its principal contaminant,  $^{82\text{m}}\text{Rb}$ , are 446, 511 and 777 keV respectively. Thus, because  $^{81\text{m}}\text{Kr}$  emits a single  $\gamma$ -ray of 190 keV it is possible to obtain the required ratio of  $^{81\text{m}}\text{Kr}$  to  $^{81}\text{Rb}$  spectroscopically. Compared with methods involving absolute uptake<sup>7,8</sup>, there are calibration advantages in using a ratio to provide a measure of tissue perfusion. The method which makes use of the ratio of  $^{81\text{m}}\text{Kr}$  to  $^{81}\text{Rb}$  is non-invasive, requiring one initial intravenous injection of  $^{81}\text{Rb}$  to make possible a series of blood flow measurements on one or more organs. Furthermore, unlike indicator dilution methods<sup>9</sup>, this technique utilizes a static measurement, thus potentially affording better statistics.

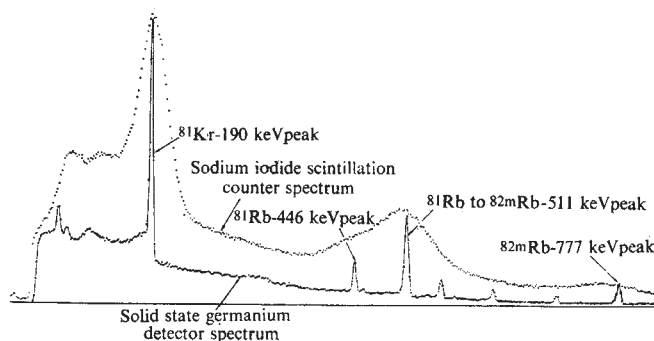


Fig. 1  $\gamma$ -Ray spectra emitted by  $^{81}\text{Rb}$  and its associated daughter  $^{81\text{m}}\text{Kr}$  and contaminant  $^{82\text{m}}\text{Rb}$  when distributed in a volume representing a heart, measured with both a sodium iodide and a lithium drifted germanium semi-conductor detector.

An important contribution to the insensitivity of this method is the fast "grow in" of the 13 s half life of  $^{81\text{m}}\text{Kr}$ . This confines its use to organs of relatively high blood flow. For example, the theoretical value of the ratio of  $^{81\text{m}}\text{Kr}$  to  $^{81}\text{Rb}$ , at a perfusion rate of 100 ml./100 g/min, is 78% of the unperfused state. In this calculation a partition coefficient for  $^{81\text{m}}\text{Kr}$  between tissue and blood of 0.9 was assumed. A further inaccuracy lies in the geometrical dependence of the ratio, necessitating equivalent measurements in a phantom if absolute perfusion values are required. The Compton distribution from the  $^{81}\text{Rb}$  and  $^{82\text{m}}\text{Rb}$   $\gamma$ -rays, especially in a distributed source, presents problems in the accurate resolution of the 190 keV  $^{81\text{m}}\text{Kr}$  peak. Fig. 1 shows two  $\gamma$ -spectra emitted by  $^{81}\text{Rb}$  and its associated daughter  $^{81\text{m}}\text{Kr}$  and contaminant  $^{82\text{m}}\text{Rb}$  when distributed in a volume representing a heart.

One was measured with a  $1.5 \times 2$  inch NaA scintillation counter and the other with a lithium drifted germanium semi-conductor detector. The sensitive volume of the latter device was  $15 \text{ cm}^3$  and the resolution equal to 4.5 keV at 667 keV. Identical collimation was provided for both detectors. This figure illustrates the need for a high resolution detector for accurate measurement of  $^{81\text{m}}\text{Kr}$  as well as the individual  $^{81}\text{Rb}$  and  $^{82\text{m}}\text{Rb}$   $\gamma$ -rays. For the simulated heart distribution the ratio of  $^{81\text{m}}\text{Kr}$  counts to the counts from scattered rubidium  $\gamma$ -rays beneath the  $^{81\text{m}}\text{Kr}$  peak was 1.46 relative to 2.92 for a point source.

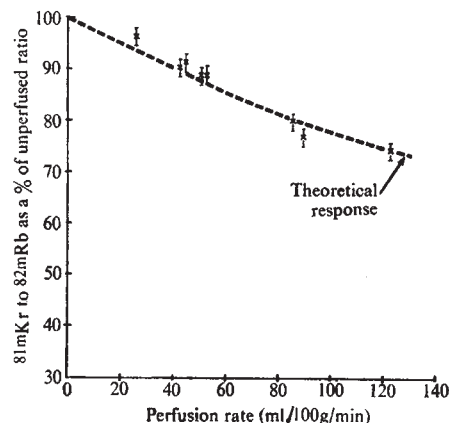


Fig. 2 Experimental correlation with the theoretical change of the ratio of  $^{81\text{m}}\text{Kr}$  to  $^{82\text{m}}\text{Rb}$  with perfusion rate.

A model of a perfused tissue in which  $^{81}\text{Rb}$  is fixed was constructed by filling the heart phantom with  $^{81}\text{Rb}$  bound to beads of 'Amberlite', a cation exchange resin (IR 120). The resin was uniformly perfused at different rates with de-ionized water and the  $^{81\text{m}}\text{Kr}$ ,  $^{81}\text{Rb}$  and  $^{82\text{m}}\text{Rb}$   $\gamma$ -rays were measured. In practice the  $^{82\text{m}}\text{Rb}$ , 777 keV  $\gamma$ -ray is used, with which to normalize the  $^{81\text{m}}\text{Kr}$ , so that the 511 keV peak is a combination of both  $^{81}\text{Rb}$  and  $^{82\text{m}}\text{Rb}$  (approximately 50%  $^{82\text{m}}\text{Rb}$  contribution, unpublished results of S. L. Waters, D. J. Silvester and I. W. Goodier), thus making decay correction inaccurate. The 777 keV  $^{82\text{m}}\text{Rb}$  peak also provides better statistics over the 446 keV  $^{81}\text{Rb}$  peak. To relate  $^{81\text{m}}\text{Kr}$  to  $^{82\text{m}}\text{Rb}$ , an assay is necessary, at a known time, of a sample of the  $^{81}\text{Rb} : ^{82\text{m}}\text{Rb}$  complex used. This follows because of the difference in half life between  $^{82\text{m}}\text{Rb}$  (6 h 18 min) and  $^{81}\text{Rb}$  (4 h 34 min).

Fig. 2 shows the experimentally obtained ratios of  $^{81\text{m}}\text{Kr}$  to  $^{82\text{m}}\text{Rb}$  expressed as a percentage of the unperfused ratio, plotted against perfusion rate. Also illustrated is the theoretical response using a partition coefficient of 0.9. The experimental data satisfactorily correlate with the theory, the deviations being due perhaps to uneven perfusion of the model.

In these conditions the sensitivity of the solid state detector was 4.6 times less than the scintillation counter. The sensitivity of the system in the conditions described was 0.084  $^{81\text{m}}\text{Kr}$  counts, 0.0093  $^{81}\text{Rb}$  (446 keV) counts, 0.044 511 keV counts and 0.027  $^{82\text{m}}\text{Rb}$  (777 keV) counts per second per  $\mu\text{Ci}$  of  $^{81}\text{Rb}$  distributed within the phantom. The estimated proportion of the activity in the heart model actually viewed was 50%.

Experimental evidence suggests that the ratio of  $^{81\text{m}}\text{Kr}$  to  $^{81}\text{Rb}$  or its  $^{82\text{m}}\text{Rb}$  contaminant could provide a quantitative index of tissue perfusion. Practical application of this method principally demands a more sensitive detector than that used for the work described here. Insensitivity is partly compensated by the static nature of the measurement. Further problems such as correction for overlying tissue, by counting a representative background area, and geometrical simulation



necessary for absolute blood flow measurements, need to be solved in order to use this technique *in vivo*.

One of the principal requirements for a technique for measuring tissue blood flow is a clinically acceptable method for studying changes in blood flow within a patient. In this context the relationship between  $^{81m}\text{Kr}$  and  $^{81}\text{Rb}$  could provide quantitative indices of tissue blood flow.

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## Acellular Corneal Transparency

THE maintenance of corneal transparency and thickness depends on the regulation of its hydration by an active metabolic process probably occurring in the endothelium<sup>1</sup>. Any inadequacy leads to overhydration, with consequent thickening and loss of corneal transparency. Although most attempts to prolong transparency in excised or cadaver corneas have been aimed at the preservation of a functioning endothelium, it has been shown that transparency can also be maintained by excision anterior to an implant of water impermeable silastic in place of the endothelium<sup>2</sup>. The critical factor thus seems to be the state of corneal hydration.

We report here that corneal transparency can be maintained in the absence of metabolic activity, by depleting the cornea of ground substance, and irradiating the stroma to stabilize the basic collagen framework.

Corneas were excised from the eyes of freshly killed cattle, sheep, pigs and rabbits, and weighed immediately, after which thickness and transparency were measured. Thickness was measured by several readings with a micrometer with contact points attached to an ohm-meter. Transparency was deter-

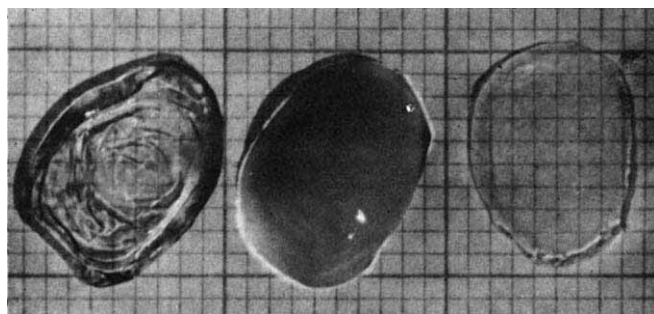


Fig. 1 (Left to right) Cow corneas immediately after removal from eye; during extraction procedure; moulded cornea after final irradiation.

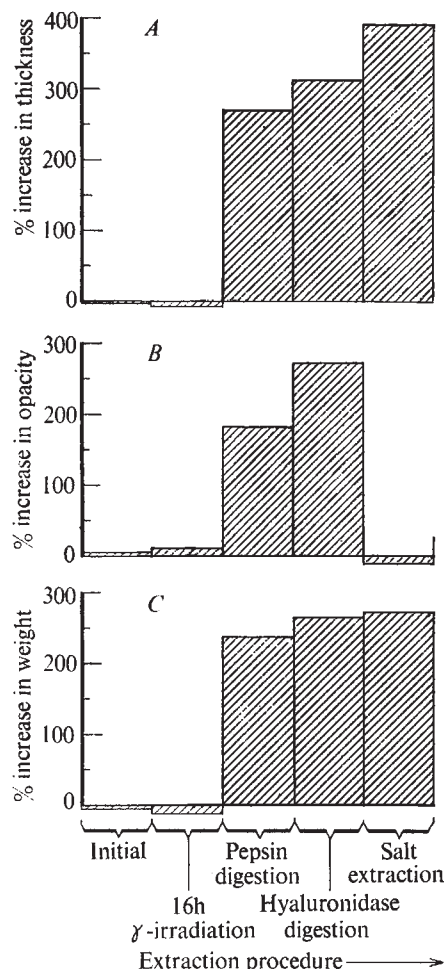


Fig. 2 Changes in thickness (A), opacity (B) and weight (C) of the animal corneas during the extraction procedure.

mined as a function of visible light transmission in a Joyce-Loebl chromoscan densitometer. Readings were repeated on corneas after each course of radiation and extraction. Corneas were first irradiated for 15 h in a  $\gamma$ -irradiator (Radiation Machinery Corp.) delivering  $1.2 \times 10^5$  rad/h. Irradiation was carried out on hydrated corneas in a nitrogen atmosphere.

Acid mucopolysaccharide ground substance and cellular debris were then extracted from the irradiated corneas. They were first incubated in pepsin, 1% of corneas wet weight, in 0.01 N HCl (pH 2) at 30° C for 2 h. The corneas were then washed and treated with hyaluronidase, 0.5% of corneal wet weight in 0.1 M  $\text{Na}_2\text{PO}_4$  in 0.15 M NaCl (pH 5.35) at 30° C for 2 h, washed, and then extracted in supersaturated solutions of  $\text{Na}_2\text{SO}_4$  with 5% NaOH for 48 h at 4° C. After a third washing, corneas were treated a second time with  $\gamma$ -irradiation in nitrogen for 16 h at  $1.2 \times 10^5$  rad/h. Some corneas were placed in moulds during this period of irradiation to preserve their spherical shape. The relative efficiency of each extraction process was judged by hexosamine determinations (Elson-Morgan)<sup>3</sup> on all supernatants and by histochemical staining of tissue sections for acid mucopolysaccharides.

The initial dosage of irradiation, given to stabilize the collagen framework before extraction, had little apparent effect on the corneas. This initial period of irradiation was omitted in some experiments and these corneas lost all semblance of structure. Some actually dissolved during extraction. Corneas rapidly opacified and swelled with the first enzyme digestion after the initial irradiation. Transparency returned promptly with  $\text{Na}_2\text{SO}_4$  extraction, although thickness and weight had increased. Hexosamine determinations on supernatants indicated that salt extractions were about five times

more effective in removing acid mucopolysaccharides than either pepsin or hyaluronidase digestion. These corneas could not be stained with alcian blue, also indicating the absence of acid mucopolysaccharides. Those corneas moulded during the final period of irradiation accepted the shape and dimension of the mould (Fig. 1).

Maintenance of corneal transparency depends on both acid mucopolysaccharide depletion and prolonged  $\gamma$ -irradiation. The doses of irradiation used were unquestionably cytotoxic, which rules out any active metabolic process. It seems likely that irradiation can introduce cross-linkages in the collagen molecules of the cornea. Transparency returns only after extraction of the bulk of ground substance with  $\text{Na}_2\text{SO}_4$  (Fig. 2). At this stage the cornea is essentially a structured membrane of hydrated collagen. Collagen membranes derived from animal hide remain permanently transparent after corneal implantation<sup>4</sup>.

Predetermined shape and thickness can be given to corneas by placing them on moulds during the second course of irradiation. We feel that this biopolymer may be useful as a prosthetic material for therapeutic corneal and intraocular lenses, and possibly as full thickness corneal replacements.

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## Removal and Separation of Flagella from Cells of *Clostridium chauvoei* and Preparation of a Pure Flagella Suspension

DURING a series of experiments at these laboratories to compare the protective antigenicity of several possible vaccine strains of *Cl. chauvoei*, one strain proved to be outstanding in protecting against a standard highly virulent challenge strain<sup>1</sup>. We have reported that guinea-pigs protected by this strain against the challenge strain show only H (or flagella) but not O agglutinating antibodies against the challenge strain. Subsequent experiments (to be published) also showed an apparent association between the H antigen and the protective antigen of this strain. One of several methods chosen to investigate this apparent association was to attempt the preparation of a pure suspension of flagella from this strain for testing as a protective antigen.

Many methods have been described for the preparation of pure flagella suspensions from a number of bacteria<sup>2,3</sup> but none of these methods was found to be satisfactory when attempted with this highly protective strain of *Cl. chauvoei*. Deflagellation was readily achieved after 3 min treatment in a Waring blender; but differential centrifugation, sucrose density gradient centrifugation and caesium chloride equilibrium density gradient centrifugation all failed to separate the deflagellated cells from the flagella. The flagella of this strain have a very strong tendency to aggregate side by side for much of their length and we thought difficulty might arise from the enmeshing of cells in a network of aggregated flagella. The following method has satisfactorily overcome this difficulty.

Whole culture, grown in a stirred fermenter vessel in digest broth medium, was deflagellated by blending for 3 min in a water-cooled Waring blender. The cells and flagella aggregates were dispersed by vibromixing the culture for 3 min in ten times the culture volume of a solution containing 0.4% NaCl and 0.02% sodium dodecyl sulphate. After this dispersal the cells were separated from the flagella by centrifugation at 16,000g for 20 min.

The supernatant was then filtered through a 'Millipore' GSWP 0.22  $\mu\text{m}$  membrane and the flagella resuspended into 0.4% saline from the surface of the membrane by gently rubbing it with a curved glass rod. The resulting flagella suspension was examined under the electron microscope and little visible contamination was evident. Platinum-carbon shadowed flagella from this suspension are shown in the electron micrograph (Fig. 1). When used to immunize rabbits, this flagella suspension stimulated an H agglutinin titre of 1 : 5120 with no detectable O response.



Fig. 1 Platinum-carbon shadowed flagella of *Clostridium chauvoei*.

No flagella were seen when the cells, recovered after centrifugation, were examined in the electron microscope. These cells failed to react with an anti-flagella (H) serum, which reacted with control (flagellated) cells to a titre of 1 : 2560. The deflagellated cells, when used to immunize rabbits, stimulated a serum H titre of 1 : 20 compared with control cells which gave an H titre of 1 : 2560.

The flagella suspension was further purified by incubating it for 3 h at 37° C in 0.05 M phosphate buffer solution (pH 7.6) containing 1 mg/ml. trypsin and 0.5 mg/ml. ribonuclease. This digestion was followed by a caesium chloride equilibrium density gradient centrifugation separation of the undigested material and yielded a clearly defined band which on electron microscopic examination was found to contain only flagella with no visible trace of contamination. Full experimental details and protection studies using the deflagellated cells and flagella suspensions will be published later.



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## Cholesterol and Hyperbaric Oxygen in Swimbladders of Deep Sea Fishes

We have found an accumulation of cholesterol within the oxygen-filled swimbladders of some deep sea benthic fishes, which suggests that certain steps in cholesterol biosynthesis and/or degradation are affected by hyperbaric oxygen. The lipid content of these swimbladders included as much as 49% (dry wt basis) of cholesterol. The lipid included major amounts of unsaturated fatty acids which resist auto-oxidative processes within the swimbladder which contains oxygen at pressures up to 380 atmospheres.

In deep sea fishes the rete mirabile gas gland is unusually large<sup>1</sup>. In fishes living deeper than 30 m the oxygen content in the swimbladders is usually constant at 90% or more<sup>2,3</sup>. Shallow water fish have a thin fatty lining within the swimbladder lumen whereas in deep sea fish this fatty lining almost fills the swimbladder.

Deep fish were caught on hooks or in traps using free vehicle methods<sup>4</sup>. Tissues were extracted on board ship with chloroform:methanol (2:1), and the lipids were weighed after drying *in vacuo*. Cholesterol and other lipids were separated by chromatography on silicic acid-'Celite' (6:1) columns eluted by increasing proportions of ether in petroleum ether. Phospholipids were eluted with methanol. Cholesterol was identified by conversion to the trifluoroacetate<sup>5</sup> and by comparison with authentic cholesteryl trifluoroacetate by gas-liquid chromatography and by Lieberman-Burchard analysis<sup>6</sup>. The composition of the gas in the swimbladder was determined using samples removed from fish under water as they arrived at the surface.

Cholesterol and phospholipids were the principal lipids in the swimbladders of these fishes. The ratio was approximately 1:1 in *Coryphaenoides acrolepis*, the Pacific rattail, and *Antimora rostrata*, the flatnose codling. In *C. abyssorum*, a rattail found only deeper than 2,500 m, there was a corresponding ratio of 1:2.5 with a considerable sterol ester fraction. Fatty acids found within these oxygen-filled swimbladders were largely, up to 82%, unsaturated (Table 1). Major amounts of oleic and palmitoleic acids as well as appreciable amounts (4.0–7.0%) 22:6 fatty acid were observed. The percentage of unsaturated acids increased at greater depths. Temperatures at these depths decrease from 6.3° C at 730 m to less than 2° C at 3,800 m. Phospholipid fatty acids did not show increased unsaturation with depth, suggesting that they are intimately involved in the structure of membrane lipoproteins<sup>7</sup>.

The high levels of cholesterol and phospholipid in swimbladders containing 90% oxygen at pressures up to 380 atmospheres suggest that oxygen or pressure effects their formation. Cholesterol biosynthesis involving squalene cyclization initiated by oxygenation to squalene-2,3-oxide may be expected to be facilitated in this oxygen-rich system. The decrease in

**Table 1** Fatty Acids of *Coryphaenoides acrolepis* Swimbladder Lipid

| Fatty acid  | Free fatty acid fraction |         |         | Phospholipid fraction |         |         |
|-------------|--------------------------|---------|---------|-----------------------|---------|---------|
|             | 730 m                    | 1,830 m | 3,800 m | 730 m                 | 1,830 m | 3,800 m |
| 16:0        | 23.1                     | 11.9    | 11.9    | 15.4                  | 11.9    | 24.1    |
| 16:1        | 4.0                      | 9.0     | 11.9    | 6.3                   | 6.4     | 16.3    |
| 18:1        | 46.6                     | 48.2    | 49.2    | 52.8                  | 48.2    | 40.3    |
| C-20, C-22  | 10.8                     | 12.6    | 18.7    | 14.9                  | 18.4    | 12.8    |
| Unsaturated | 63.5                     | 73.5    | 82.2    | 79.3                  | 80.0    | 72.7    |

The fatty acid is designated by the number of carbon atoms and the number of double bonds. C-20, C-22 refer to the total percentage of 20 and 22 carbon fatty acids. Unsaturated means the total percentage of unsaturated fatty acids, including 16:1 and 18:1. Analyses, in triplicate, are given for fishes collected at the depths indicated. Standard deviations were less than 0.5%.

molar volume in the transition from squalene to cholesterol may also accelerate the cyclization process. The presence of cholesterol and phospholipid in nearly equal amounts as is found in myelin and erythrocytes suggests that membranes may be involved in their formation or transport. The swimbladder contents of *C. acrolepis* included 18% (dry wt basis) protein which may originate from the membranes.

The consistent and abundant presence of cholesterol and phospholipid in all the swimbladders of the deep sea fish we examined may relate to some mode of buoyancy control. The solubility of oxygen in this lipid and lipoprotein may affect their densities or facilitate the secretion of gas at great pressures in the ocean.

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## Leucocytosis in the Mouse induced by Serial Blood Sampling from the Tail

THE simplest and most widely used method for blood sampling in the mouse is to snip off the tip of the tail with scissors<sup>1</sup> but the effect of serial tail snipping on white blood corpuscle count has not, as far as we know, been reported in the literature.

Three groups of five mice were used and leucocyte counts were made every 2 h by taking 5 µl. of blood from the snipped tail. The amount of blood is so small that it does not influence the regeneration of blood corpuscles in the bone marrow. Male mice were used to avoid changes due to the oestrous cycle and each experiment was started at a different hour of day to exclude the effect of diurnal variation<sup>2</sup>. Leucocytes were counted in a Bürker-Türk chamber. Differential leucocyte counts were examined in blood smears taken every 4 h from the animals of one group. Smears were stained with May-Grünwald-Giemsa stain and 300 cells were counted.

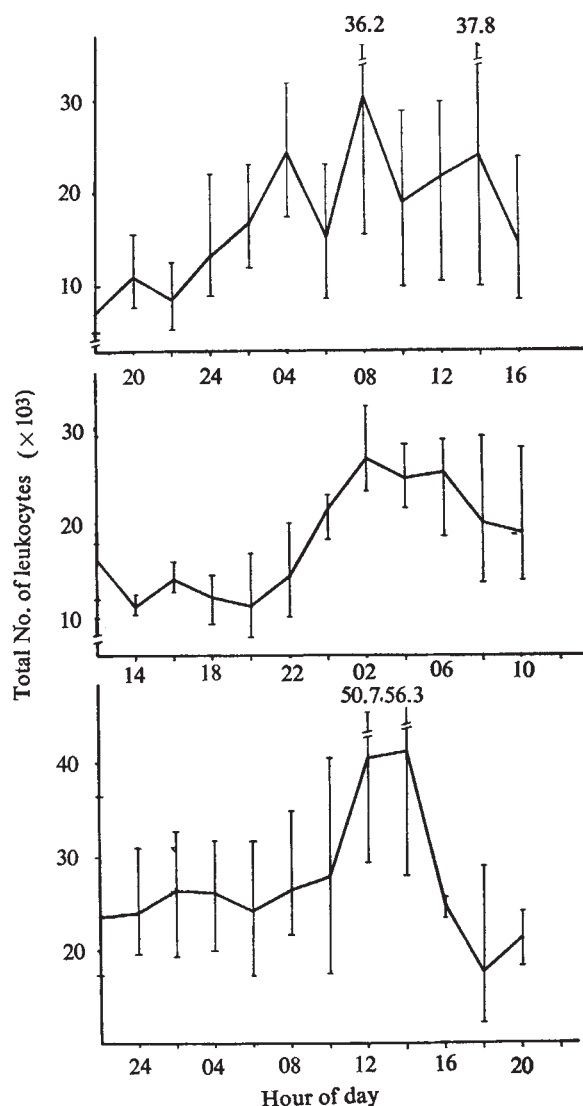


Fig. 1 Leucocyte counts for three groups of five mice each as a function of time.

In each group, a marked leucocytosis was observed 10 h after the beginning of the experiment (Fig. 1). In the control group, one sample only was taken 10 h after the first sample, and no leucocytosis was found. A decrease in the relative number of the mononuclear cells and an increase in the number of granulocytes were observed.

The leucocytosis was no doubt induced by serial blood sampling by tail snipping. The mechanism is unknown. Inflammation is not probable because leucocyte counts decreased quickly after the peak. Leucocytosis can be induced in certain limits by adrenal cortical hormones, which are known to cause a decrease in the mononuclear cell count and an increase in the granulocyte count<sup>3</sup>. The microtechnique of blood sampling is inaccurate and its use necessitates rather extensive variations in blood corpuscle counts.

The present study indicates that the micro-scale blood sampling from the mouse tail can induce a marked leucocytosis, which must be noted in leucocyte counting.

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## Possible Mechanism of Egress of Free Cholesterol from the Arterial Wall

SPERRY<sup>1</sup> was the first to demonstrate *in vitro* esterification of cholesterol, reporting that incubation of human serum and plasma at 37° C for 3 days decreased free cholesterol without changing total cholesterol. He also showed that esterification of free cholesterol during incubation could be inhibited by heating serum to 55°–60° C for 1–2 h, and suggested that this procedure resulted in enzyme inactivation. Glomset and Wright<sup>2</sup> showed a similar inhibition of cholesterol esterification after heating serum for 30 min at 56° C; this has been confirmed by others<sup>3,4</sup> as well as by us. There is evidence that the heat-labile enzyme is lecithin-cholesterol-acyl-transferase (LCAT) which seems to react preferentially with high density lipoproteins to catalyse the transfer of fatty acids from the 2-position of lecithin to the hydroxyl group of free cholesterol. Murphy<sup>4</sup> has demonstrated an equilibrium between free cholesterol of serum and that of the red blood cells, and shown that the flux of free cholesterol from cells to serum increases as the free cholesterol of the serum becomes esterified during incubation. If there was a similar equilibrium between free cholesterol of the serum and of the arterial tissue, the rate of esterification of free cholesterol might be an important factor in similarly allowing more cholesterol to leave the arterial wall. To test this hypothesis segments of human iliac arteries which appeared from gross examination to be free of atherosclerosis were removed at autopsy in sterile conditions, washed with cold saline, stripped of adventitia and the wall turned inside out, so that the intimal surface was then external, and the ends were sewn together<sup>6</sup>. These arterial segments were used immediately or stored frozen and dried in sterile tubes until needed. Fasting blood samples were drawn from healthy volunteers, without the use of anticoagulants, defibrinated and centrifuged at 3,000g for 10 min. The serum was removed and used immediately for all incubation procedures. The serum was first separated into two large aliquots, one of which was heated at 56° C for 30 min to inactivate LCAT enzyme. Samples of both untreated and inactivated serum were incubated for 6 h at 37° C. The decrease in serum free cholesterol concentration recorded after incubation was a measure of LCAT activity. Inactivation of serum by heating always prevented a decrease in the free cholesterol during incubation. Three iliac arterial segments, equal in size and area, and removed from the same necropsy and prepared as described, were used for subsequent incubation experiments. One segment was first heated at 56° C for 30 min to inactivate any LCAT enzyme from the arterial wall and the other two were untreated. Incubations for 6 h at 37° C were then carried out in the following conditions: 5 ml. aliquots of serum (untreated) were incubated with arterial segments (untreated); a similar untreated aliquot of serum was incubated with an inactivated arterial segment, and an aliquot of inactivated serum was incubated with an untreated arterial segment. Incubations were carried out in small sealed Erlenmeyer flasks to which oxygen had been added. The flasks were placed in a Dubnoff shaker to mix the serum and arterial intima. At the end of incubation arterial segments were carefully lifted and any serum adhering to the intima was allowed to drain off for 15 min. The total and free cholesterol concentrations were then determined by the method of Zak *et al.*<sup>7</sup> on all serum samples for all the conditions described. The data on initial LCAT activity could be used as a measure of the expected decrease in free



cholesterol with incubation of serum alone, and any increase of total and free cholesterol in the serum after incubation with arterial segments could be taken as a measure of egress of cholesterol from the arterial wall (Table 1). The decrease in free cholesterol when serum was incubated alone (A) occurs without any significant change in total cholesterol, the slight difference being compatible with a less than 2% error in the technique. Furthermore, the method of inactivation of the LCAT enzyme of the serum was dependable, the decrease in free cholesterol being negligible (B). Whenever arterial segments were incubated with serum, there was an increase in the mean total cholesterol concentration (C, D, E) regardless of prior inactivation. When serum was inactivated before incubation with the arterial wall (C) there was no change in the concentration of free cholesterol, which increased, however, after incubation of untreated serum regardless of any prior inactivation of the arterial wall (D, E). The lower part of Table 1 shows that there was a similar egress of total and free cholesterol from the arterial wall after incubation with untreated serum. The negligible increase of free cholesterol in the inactivated serum (C-B) is significantly different from the other two conditions at the  $P < 0.001$  level. But there are no significant differences in the increase in total cholesterol for all the groups. Thus when LCAT enzyme is absent from the incubation medium, only cholesterol ester seems to leave the arterial wall, whereas when LCAT enzyme is present, virtually only free cholesterol seems to leave the arterial wall.

**Table 1** Mean Changes in Total and Free Cholesterol (mg/100 ml.)

|                                                                                     | Total cholesterol $\pm$ s.d. | Free cholesterol $\pm$ s.d. |
|-------------------------------------------------------------------------------------|------------------------------|-----------------------------|
| (A) Serum at 37° C (LCAT activity)                                                  | 3.07 $\pm$ 7.27              | -12.67 $\pm$ 4.79           |
| (B) Serum at 37° C after 0.5 h at 56° C (inactivated LCAT)                          | -0.20 $\pm$ 3.70             | -0.67 $\pm$ 2.44            |
| (C) Inactivated serum + arterial wall at 37° C                                      | 12.53 $\pm$ 10.75            | +0.20 $\pm$ 2.70            |
| (D) Serum + arterial wall at 37° C                                                  | 12.13 $\pm$ 11.96            | -5.07 $\pm$ 3.83            |
| (E) Serum + inactivated arterial wall (0.5 h at 56° C) at 37° C                     | 9.53 $\pm$ 8.96              | -5.73 $\pm$ 3.08            |
| Difference in concentrations of cholesterol in serum with and without arterial wall |                              |                             |
| D-A                                                                                 | 9.06 $\pm$ 2.03              | 7.60 $\pm$ 0.98             |
| E-A                                                                                 | 6.46 $\pm$ 2.03              | 6.94 $\pm$ 0.98             |
| C-B                                                                                 | 12.73 $\pm$ 2.03             | 0.87 $\pm$ 0.98 *           |

Plasma from fifteen individuals was incubated for 6 h in each condition.

\* Significantly different from other two conditions at  $P < 0.001$  level.

Our results suggest that a decrease in serum free cholesterol, caused by a serum cholesterol esterifying enzyme, possibly LCAT, might be important in promoting the removal of free cholesterol from the arterial wall. This mechanism would be consistent with the finding (refs. 8 and 9 and unpublished work of H. L. R., A. G. Stern, L. A. S., and S. deB. Braverman) that there is a tendency for LCAT activity in the serum to be higher in subjects with hypercholesterolaemia greater than 300 mg/100 ml. which protects the arterial wall from excessive free cholesterol. It is also consistent with other studies in which serum was necessary for the release of  $^{14}\text{C}$ -cholesterol from rabbit aortae<sup>10</sup> and for the efflux of labelled cholesterol across the intimal surface of rat aortae<sup>11</sup>. The latter study showed increased egress of free cholesterol from the arterial wall, whether boiled or not, but the effects of

inactivated serum were not determined; and the former study, though demonstrating increased egress of cholesterol esters, used aortae which were halved and which exposed portions other than the intima to the surrounding medium; furthermore, rabbit aorta and serum were used rather than human aorta and serum as we have done, and Bailey<sup>12</sup> has reported less excretion of cholesterol by tissue culture cells incubated in rabbit serum compared with those incubated in human adult serum. We have also corroborated studies<sup>6,10,11</sup> which showed that boiling of the arterial tissue did not decrease the egress of cholesterol from the aorta into the serum, a finding compatible with the concept that viable cells and enzymatic processes within the arterial wall are unnecessary for efflux to occur.

**Table 2** Change in Total and Free Cholesterol (mg %) in the Incubation Medium after Heating at 37° C for 6 h

|              | Serum + artery |     | Glucose-saline + artery |    | Glucose-saline + inactivated artery |    |
|--------------|----------------|-----|-------------------------|----|-------------------------------------|----|
|              | TC             | FC  | TC                      | FC | TC                                  | FC |
| Experiment 1 | +14            | +17 | 0                       | 0  | 0                                   | 0  |
| Experiment 2 | +18            | +10 | 0                       | 0  | 0                                   | 0  |

Cholesterol in medium after incubation of arterial segments at 37° C

| No. of determinations                            | 6 h incubations  |  | Glucose-saline | Krebs-Ringer phosphate buffer |
|--------------------------------------------------|------------------|--|----------------|-------------------------------|
|                                                  | 24 h incubations |  |                |                               |
|                                                  |                  |  | 4              | 1                             |
|                                                  |                  |  | 2              | 1                             |
| Cholesterol concentrations in each determination |                  |  | 0              | 0                             |

Incubations were performed simultaneously on segments of the same artery.

TC, Total cholesterol; FC, free cholesterol.

We cannot explain the egress of only cholesterol ester from the arterial wall in the presence of inactivated serum. Possibly *in vitro* the total exchange after incubation reflects other important factors or enzymes of the arterial wall which would not be involved in the presence of LCAT as well as any other cholesterol esterifying agent. Thus, Abdulla *et al.*<sup>13</sup> reported LCAT activity in homogenized human and rabbit aortae, mostly in the outer part of the atherosclerotic intima, which could be partly due to the hyperplastic layer of smooth muscle cells in that zone<sup>14</sup>. They suggested that more polyunsaturated cholesterol esters were excreted from the vascular wall than free cholesterol, but they based these suggestions primarily on differential absorption rates of subcutaneous implants of labelled cholesterol and cholesterol esters<sup>15</sup>. Other differences between our results and those of Abdulla *et al.* could be due to the fact that we used intact aorta rather than homogenized tissue. LCAT activity deeper in the vascular wall might not have an effect on the serum exposed only to the lumen of the vessel in our experiments. Incubation of arterial segments with buffer solution or glucose and saline in our laboratory has revealed no significant egress of any form of cholesterol into the medium (Table 2), so that egress of only cholesterol ester after inactivation of serum could be caused by an agent in the serum other than LCAT and which is operative only, or maximally, when LCAT is absent. Others have also reported negligible excretion of labelled cholesterol into saline or Krebs-Ringer phosphate buffer media from, first, tissue culture cells<sup>12</sup> and from rat aortae<sup>11</sup>. Both studies concluded that cholesterol excretion is dependent on or stimulated by the presence of serum, serum proteins or lipoprotein solution.

Our results may be particularly important because we have recently found cholesterol esterification to be significantly

impaired in subjects with acute myocardial infarction and chronic coronary artery disease when compared with age matched controls. Thus, a deficiency of cholesterol esterifying enzyme, possibly LCAT, may be important in the aetiology or rate of development of atherosclerosis.

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## Diagnosis of Resistance to Organophosphorus Insecticides in *Myzus persicae* (Sulz.)

THE chemical control of the green peach aphid *Myzus persicae* (Sulz.), which damages many crops both directly and by transmitting numerous virus diseases, is seriously jeopardized by the world wide occurrence of resistance to organophosphorus insecticides. In the United Kingdom resistance previously confined to glasshouse crops now occurs in the field. Nothing is known about the nature of the resistance mechanism(s) in these insects and little about the patterns of resistance. We wish to report (1) large differences in the activity of ali-esterases of susceptible and resistant aphids which can be used to determine resistance even in single aphids, (2) large differences in some properties of these enzymes between susceptible and resistant aphids and house flies, and (3) a marked decrease or increase in resistance when the aphids are treated with insecticides in the presence of 'Sesamex' (2-(3,4-methylene-dioxyphenyl)3,6,9-trioxadecane). This is evidence that multi-function oxidases are involved in resistance to organophosphorus insecticides in aphids.

The GR strain of *Myzus persicae*, on which most of the work was done, was derived from a glasshouse population that had become resistant to organophosphorus insecticides after exposure to many insecticides (including organophosphorus insecticides and carbamates)<sup>1-3</sup>. The strain was exposed continuously to and selected with dichlorvos from a 'Vapona'

plastic strip. Bioassays with insecticides applied topically<sup>4</sup> showed that the GR strain was very resistant to parathion, dimethoate, disulphoton, oxydisulphoton and "disulphoton sulphone" ((C<sub>2</sub>H<sub>5</sub>O)<sub>2</sub>PS.S(CH<sub>2</sub>)<sub>2</sub>SO<sub>2</sub>.C<sub>2</sub>H<sub>5</sub>; Table 1), but only about ten times more resistant to demeton-S than the susceptible strain and slightly resistant to the carbamate pirimicarb (twelve times). A sub-culture (GRUS) of the resistant strain, started three months after receipt of the GR strain and bred in conditions free from insecticides, retained the great resistance to dimethoate of the selected parent for about fourteen generations, but lost it rapidly, for unknown reasons, during the next four generations when its resistance to dimethoate dropped to four times that of the susceptible strain.

**Table 1** Resistance Factors \* at LD<sub>50</sub> of the GR Strain of Organophosphorus Resistant *Myzus persicae* to Various Insecticides

|                        |          |
|------------------------|----------|
| Dimethoate             | 212†     |
| Parathion              | 171†     |
| Disulphoton            | 75†      |
| "Disulphoton sulphone" | 398      |
| Oxydisulphoton         | 457      |
| Demeton-S              | About 10 |
| Pirimicarb             | 12†      |

\* Resistance factor =  $\frac{\text{LD}_{50} \text{ of resistant strain}}{\text{LD}_{50} \text{ of susceptible strain}}$

† Mean values.

**Table 2** Hydrolysis of  $\alpha$ -Naphthyl Acetate by Several Strains of *Myzus persicae*

| Strain                   | Origin                      | Amount of $\alpha$ -naphthyl acetate hydrolysed in 30 min (mol/mg aphid $\times 10^8$ ) | Response of strains to OPs |
|--------------------------|-----------------------------|-----------------------------------------------------------------------------------------|----------------------------|
| Rothamsted (susceptible) | Rothamsted                  | 1.4                                                                                     | Susceptible                |
| GR (resistant)           | UK *                        | 8.6                                                                                     | Very resistant             |
| GRUS (resistant)         | Unselected sub-strain of GR | 8.4                                                                                     | Very resistant             |
|                          |                             | 2.3                                                                                     | Slightly resistant         |
| Ferrara                  | Italian †                   | 14.9                                                                                    | Resistant                  |
| East Malling             | UK ‡                        | 4.7                                                                                     | Moderately resistant       |
| East Malling             | UK §                        | 4.2                                                                                     | Resistant                  |

\* Obtained from H. J. Gould, National Agricultural Advisory Service, Reading.

† Obtained from Dr F. Baranyovits from peach trees in the Ferrara district where phosdrin gave very poor control.

‡ Obtained from Dr G. H. L. Dicker, East Malling Research Station. Collected from an infestation on outdoor-grown peaches which was not controlled by dimethoate and malathion.

§ Obtained from Dr G. H. L. Dicker. Collected from glass-house tomatoes after failure of malathion to control aphids.

The ali-esterases of the susceptible and resistant aphids differed greatly in their ability to hydrolyse  $\alpha$ -naphthyl acetate, the exact reverse of the activities reported for strains of house flies susceptible and resistant to organophosphorus insecticides. The hydrolysing activity of single aphids is easily measured by van Asperen's colorimetric method<sup>5</sup>, and Table 2 compares the activity of several strains of aphids. All the organophosphorus resistant strains tested had high ali-esterase activity, and the loss in resistance in our unselected strain was reflected by a corresponding loss of activity, indicating that in aphids resistance to organophosphorus insecticides is linked with high ali-esterase activity.

The aphids and house flies also differ in the distribution of the ali-esterase activity between sub-cellular fractions. Centrifuging the 30,000g supernatant fluid for 4 h at 100,000g gave two fractions, the "supernatant" and the "pellet", which were examined separately for activity in susceptible and resistant



**Table 3** Ali-esterase Activities of 100,000g "Supernatant" and "Pellet" of Susceptible and Resistant Aphids and House Flies

| Sub-cellular fraction | Ali-esterase activity* |           |             |           |
|-----------------------|------------------------|-----------|-------------|-----------|
|                       | Aphids                 |           | House flies |           |
|                       | Susceptible            | Resistant | Susceptible | Resistant |
| 100,000g supernatant  | 0.13                   | 1.45      | 0.39        | 0.20      |
| Pellet                | 0.32                   | 0.43      | 0.38        | 0.060     |

\* Activities expressed in mol of  $\alpha$ -naphthyl acetate hydrolysed after 30 min/mg insect  $\times 10^8$ .

aphids and house flies (Table 3). It is clear that whereas susceptible and resistant strains of aphids differ in the activity of their supernatant fractions, house flies differ mainly in the activity of the pellets. The ali-esterase of aphids also seems less sensitive than the ali-esterase of house flies to inhibition by organophosphorus insecticides. Resistant and susceptible aphids treated with 0.25  $\mu$ l. of 0.2% w/v parathion (slightly more than the LD<sub>50</sub> of the resistant aphids) had almost the same ali-esterase activity 2 h after treatment as the untreated aphids even though all the susceptible aphids were then apparently dead. Under comparable conditions house fly ali-esterase would have been completely inhibited. Thus, although the activity of the ali-esterase can be used to determine resistance to organophosphorus insecticides in both house flies and aphids, the ali-esterases of these two species of insect differ greatly.

The difference in ali-esterase activity is not confined to organophosphorus-resistant house flies and aphids. Malathion resistance in the blowfly *Chrysomya putoria* (Wild) is linked with low ali-esterase activity (substrate  $\alpha$ -naphthyl acetate), whereas resistance to several organophosphorus insecticides in the hemipterans, the green rice leafhopper *Nephotettix cincticeps* Uhler<sup>8</sup> and the small brown leafhopper *Laodelphax striatellus* Fallen<sup>8,9</sup>, is linked with high ali-esterase activity (substrate  $\beta$ -naphthyl acetate). The colorimetric test for increased ali-esterase activity developed by Ozaki<sup>8</sup> to detect organophosphorus resistance in the rice leafhopper could, in a modified form, be used to detect resistance in aphids. It would be of use only where resistance to organophosphorus insecticides is linked with a change in ali-esterase activity.

To determine further the nature of the resistance mechanisms to organophosphorus insecticides in aphids, 'Sesamex', which is a multi-function oxidase inhibitor<sup>10</sup>, was incorporated in the solutions of insecticides applied to the aphids. 'Sesamex' reduced the resistance to dimethoate from 200 times to three times, but increased resistance to disulphoton from seventy-five times to almost complete immunity. This contrast in response to these two insecticides in the presence of 'Sesamex' may be because 'Sesamex' in one case blocks the mechanism that detoxifies dimethoate, while in the other it prevents the conversion of disulphoton to demeton-S to which aphids are only slightly resistant (Table 1). This suggestion that multi-function oxidases play an important role in the resistance of aphids to organophosphate insecticides is now being investigated. The suppression of resistance to dimethoate suggests that synergists with translaminar or systemic properties could have considerable practical value in overcoming resistance to some of the more widely used systemic insecticides.

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## Attenuation of Stimulus Sensitivity induced by Scopolamine

SEVERAL studies have shown that scopolamine hydrobromide, a peripheral cholinergic blocking agent<sup>1</sup>, increases responding in situations which require response suppression<sup>2,3</sup>. Other studies<sup>4</sup> have demonstrated similar behavioural effects when cholinergic function was modified by anticholinesterases. This disruption has been attributed to either impairment of sensory input<sup>2</sup> or response disinhibition<sup>5</sup>. These hypotheses were tested by using a form of analysis based on the theory of signal detectability (TSD), which originated in work on electronic communication systems and has been extended to sensory psychology<sup>6</sup>. It has been particularly useful in psychophysics because it yields independent estimates of a subject's sensitivity to stimuli and his response bias.

We have applied the TSD analysis to performance in a discrete trial situation involving response suppression. A hungry animal was reinforced with food only if his responses were made during the 15 s for which a cue light was on while responses at other times reset the 15 s intertrial interval. The cue light increased the illumination in the experimental chambers to 0.30 lamberts compared with 0.10 lamberts background illumination giving a  $d' = 3$  theoretically.

Three groups of four adult male albino (Wistar) rats, housed individually, were maintained at either 75%, 80%, or 85% of their "free feeding" weight. They were trained to lever press in a Grason-Stadler (type E 3125 B) operant box using 45 mg Noyes pellets of food. Thereafter they were reinforced only for a response during a trial while response at other times reset the intertrial interval. Supplements of 15 g of dry laboratory diet were given after each daily session. They were maintained on the schedule for forty-five daily sessions until the behaviour was stable for 40 days in order to satisfy the TSD assumption of well practised observers, that is, subjects that have an ordinal scale of likelihood ratio that a particular event was caused by a signal rather than noise<sup>6</sup>.

After stable performance had been established, doses of 0, 0.0625, 0.125, and 0.25 of scopolamine hydrobromide were injected intraperitoneally on alternate days, according to a latin-square design, provided that performance returned to the baseline level on the intervening day. Previous studies had shown that this interval between injections was sufficient<sup>3</sup> and the data for this experiment confirmed this.

The experimental results were recorded in terms of the number of responses, the number of reinforcements and the inter-response times (IRTs) using ten 3 s intervals. From the IRT data, estimates were made of the probability of responding in a particular time interval provided that the animal reaches the initial boundary of that interval and so has an opportunity of responding during the interval. The "false alarm" rate,  $p(S/n)$ , was calculated for the 3 s interval immediately before reinforcement was available. The data for the other intervals were not used in the calculation because some of these represented post-reset "bursts" of responding which may depend on factors such as deprivation conditions, apparatus, and so forth<sup>7</sup>.

The probability of a response for the interval between 16 and 18 s was computed to give the "hit" rate,  $p(S/s)$ .

There were no significant differences between the different motivational levels so that it was not possible to plot an ROC curve. Instead the false alarm and hit rates for each animal for each dose level were plotted on double probability graph paper and an index of sensitivity,  $d'_e$ , was estimated for each one by the procedure suggested by J. P. Egan and described by Green and Swets<sup>6</sup>. The mean  $d'_e$  for the three groups is plotted as a function of dose level (Fig. 1). It is clear that there is a decrease in sensitivity for all groups as the dose level increases. There was no overlap between the indices for the 0 and 0.0625 mg/kg dose levels, and no overlap between 0.0625 and 0.125 with the 0.25 mg/kg doses. A Wilcoxon matched pairs signed-ranks test<sup>8</sup> shows that there were no significant differences ( $P > 0.05$ ) between the 0.0625 and 0.125 mg/kg dose levels. We are not aware of any study that has used and detected a dose as low as 0.0625 mg/kg indicating the sensitivity of the TSD analysis. These changes in sensitivity were not accompanied by any lowering of the animal's response criterion; rather any shift was in the direction of an increase in criterion, giving fewer "hits" but also fewer "false alarms".

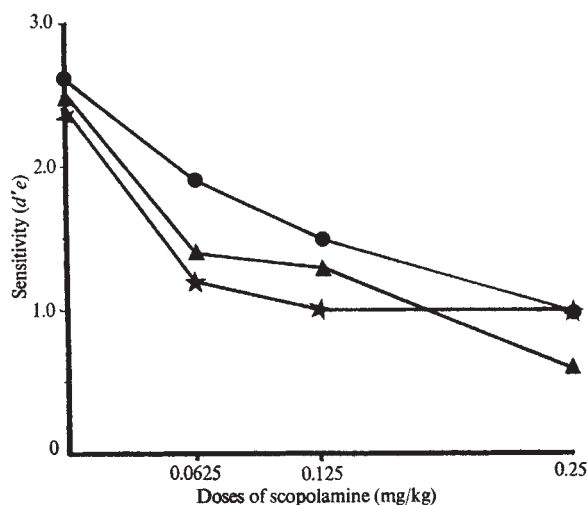


Fig. 1 Changes in stimulus sensitivity,  $d'_e$ , after injections of scopolamine for three groups at different motivational levels. ●, 85%; ▲, 80%; ★, 75%.

These results clearly indicate that the drug modifies behaviour by reducing the signal to noise ratio and not by lowering the animal's response criterion. The absence of any decrease in the response criterion contradicted the hypothesis that the scopolamine-induced disruption was the result of response disinhibition<sup>5</sup>. In perceptual theory, attention has been interpreted as a process of selection produced by increases in the signal to noise ratio in sensory systems<sup>9</sup>, and impairment of attention would be expected to produce a decrease in sensitivity with little consistent shift in the criterion<sup>10</sup>. Thus our results are consistent with the hypothesis that scopolamine disrupts attention mechanisms by impairing the ascending cholinergic reticular pathways<sup>11</sup>.

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## Conjugation Defect in Tyramine-sensitive Migraine

IN about 30% of patients with classical migraine, headaches can be provoked by tyramine and tyramine containing foods<sup>1</sup>. There is some evidence that ingested tyramine gains access to the circulation of subjects with such dietary migraine more readily than to that of controls, although the mechanisms by which it triggers a migraine attack remain largely speculative.

Most authors assume that tyramine degradation takes place solely by oxidative deamination catalysed by monoamine oxidase (MAO)<sup>3-5</sup>. We have found evidence for a decrease in platelet MAO activity during headache episodes; but the platelet variant may not be representative of MAO in other sites<sup>8</sup>, and we present evidence here that *in vivo* MAO activity is not impaired. Conjugation is a quantitatively minor tyramine inactivation mechanism which may be impaired in tyramine-sensitive migraine<sup>9</sup>. In this communication, we confirm and extend this finding and show that it may be one manifestation of a generalized conjugation defect.

Eight female patients with tyramine-sensitive migraine and twelve normal adult volunteers (seven male, five female) emptied their bladders and then swallowed capsules containing either 125 mg of tyramine HCl followed by 125 mg of lactose on the following day or vice versa. The order was randomized. Urine collections were made for 3 h after ingestion of each capsule because most metabolized tyramine is excreted within 3 h<sup>10</sup>. Specimens were acidified to about pH 2 with 6 N HCl before storage at  $-15^{\circ}\text{C}$ .

Samples were analysed for free and conjugated tyramine by the method of Oates<sup>11</sup> before and after hydrolysis with a sulphatase-glucuronidase preparation<sup>12</sup>. Free *p*-hydroxyphenylacetic acid was quantified by the gas chromatographic procedure of Karoum *et al.*<sup>13,14</sup>. Output of total metadrenalines and 4-hydroxy-3-methoxyphenylglycol (HMPG) was measured by the method of Ruthven and Sandler<sup>12</sup>.

Tyramine excretion values are shown in Table 1. After an oral tyramine load, there was a significantly greater increase in free tyramine and a decrease in conjugated tyramine in migraine compared with control subjects. Apart from the unexplained discrepancy in output of free tyramine after an oral load in our respective groups of migraine subjects, our findings are in line with the data of Smith and co-workers<sup>9,15</sup>. The reason for the paradoxically low output of free tyramine in migraine subjects compared with controls after placebo ingestion is not immediately obvious.

The output of two urinary constituents, total metadrenalines and HMPG, which are normally excreted largely in conjugated form<sup>16,17</sup> (Table 2) gave some indication of whether the conjugation defect is restricted to tyramine. A significant decrease in metadrenaline excretion, and a decrease in HMPG which did not, however, reach significance, was noted in migraine subjects. Analytical data are presented for placebo urines only; the pattern after tyramine ingestion is complicated by its ability to release noradrenaline from binding sites and so



**Table 1** Urinary Tyramine after Ingestion of Tyramine Hydrochloride or Lactose

| Group    | N  | Free          | Lactose       |               | Tyramine hydrochloride (125 mg) |               |               |
|----------|----|---------------|---------------|---------------|---------------------------------|---------------|---------------|
|          |    |               | Conjugated    | Total         | Free                            | Conjugated    | Total         |
| Migraine | 8  | 0.077 ± 0.015 | 0.355 ± 0.074 | 0.432 ± 0.088 | 2.930 ± 1.094                   | 2.047 ± 0.401 | 4.987 ± 0.746 |
| Control  | 12 | 0.141 ± 0.023 | 0.408 ± 0.018 | 0.549 ± 0.041 | 0.991 ± 0.214                   | 9.108 ± 1.068 | 10.01 ± 1.187 |
| P        |    | <0.02         | NS            | NS            | <0.05                           | <0.001        | <0.01         |

Urinary output (mean ± s.e.) of tyramine (mg/3 h) in patients with dietary migraine and control subjects after oral dosage with tyramine hydrochloride, compared with excretion values after lactose ingestion (NS, not significant).

increase the output of such noradrenaline metabolites as the metadrenalines and HMPG<sup>2</sup>.

There was no evidence of any generalized *in vivo* defect in oxidative deamination in affected subjects as signified by the normal output of *p*-hydroxyphenylacetic acid, the chief metabolite of tyramine, in migraine subjects (Table 3).

Although the role of conjugation in the biological disposition of tyramine in the human had been largely overlooked, it is known to play a part in the metabolism of other biologically active monoamines. Some workers<sup>8,19</sup> have shown that a proportion of ingested adrenaline is conjugated, while we have demonstrated<sup>20</sup> that isoprenaline, which is not a substrate for MAO, is largely metabolized by conjugation.

**Table 2** Urinary Metadrenalines and HMPG

|          | N  | Total metadrenalines | HMPG     |
|----------|----|----------------------|----------|
| Migraine | 8  | 42.4 ± 3.6           | 227 ± 53 |
| Control  | 12 | 73.3 ± 6.0           | 322 ± 29 |
| P        |    | <0.001               | NS       |

Urinary output (mean ± s.e.) of total metadrenalines and 4-hydroxy-3-methoxyphenylglycol (HMPG) (μg/3 h) in patients with dietary migraine and control subjects (NS, not significant).

**Table 3** Urinary *p*-Hydroxyphenylacetic Acid after Ingestion of Tyramine or Lactose

|          | N | Lactose       | Tyramine hydrochloride (125 mg) |
|----------|---|---------------|---------------------------------|
| Migraine | 4 | 1.023 ± 0.272 | 44.49 ± 24.98                   |
| Control  | 4 | 1.368 ± 0.411 | 41.85 ± 20.88                   |

Urinary output (mean ± s.e.) of *p*-hydroxyphenylacetic acid (mg/3 h) in patients with dietary migraine and control subjects after oral tyramine ingestion compared with lactose.

We suspect that the enzyme defect we have investigated is one of sulphate conjugation, for both metadrenalines and HMPG are predominantly excreted in man as sulphates<sup>16,17</sup>. There is some direct evidence to support this view<sup>15</sup>. Further investigations with other substrates of this enzyme system are obviously necessary, together with direct examination of biopsy samples of jejunal mucosa in affected and control subjects. Because it is possible that we are dealing with a pharmacogenetic disease<sup>21</sup>, family studies are needed.

Whatever the nature of the biochemical lesion, it has at least two important implications for clinical practice. Patients being treated with MAO inhibiting drugs<sup>4,5</sup> normally possess in conjugation a "safety valve" alternative pathway for the degradation of tyramine; its absence in certain individuals might make such therapy dangerous for them. Further, conjugation is the most important metabolic pathway for the disposition of oral isoprenaline<sup>20</sup>. Any attenuation of this enzymatic mechanism might render isoprenaline therapy extremely hazardous. Attention has been drawn to an increasing number of deaths from an unknown cause in young

asthmatics after the use of isoprenaline inhalers<sup>22,23</sup>. We suggest that a conjugation defect of the type we have described may be a contributory factor.

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# BOOK REVIEWS

## Lessons in Management

*Mid-Career Development: Research Perspectives on a Developmental Community for Senior Administrators.* By Robert N. Rapoport. With contributions by M. B. Brodie and E. A. Life. Pp. xiii+290. (Tavistock: London and New York, 1970.) £2.90.

THE Administrative Staff College at Henley was one of the earliest British entrants (1948) into management education; it had 15 years of pioneering work before the business schools were born. This very careful analysis of its experience is therefore of first-class importance, and is full of lessons for what is now a great industry.

Henley concentrated on a 3 month course, teaching as wide a range of managers as it could get from private manufacturing industry, commerce, banking and services, nationalized industry, central and local government. The average age was 39, about half of them graduates, and the median salary pre-Henley (1967 prices) around £3,200, and post-Henley about £3,800. These are mid-career courses, aimed at enabling men to take the jump to the posts that lead to higher management.

The book is about what one can only describe as a great research project by the College's research staff and the Tavistock Institute. They collected returns from 576 people who were at Henley from 1960 to 1966, some 70 per cent of all those who were there—it seems a remarkable achievement to have obtained replies from so many and to such a huge questionnaire. Of these, 52 per cent were from private industry, 14 per cent from nationalized industry, 15 per cent from commerce and banking and 19 per cent from central and local government. The questionnaire showed how they came to go to Henley, what they did there, how they gained from their experience and how they have fared since. This is unique material for everyone engaged in this field.

There were no great surprises. 57 per cent thought they developed by "a fair amount" or more as a result of the course: the typical member was promoted a year or two after attending the course (and the pay of the median increased by about 20 per cent at 1967 prices): 70 per cent recorded an increase afterwards in the number and magnitude of decisions taken without higher authority. They all reached remarkable agreement on management philosophy, 93 per cent agreeing that "the most important skill for managers in future is planning and controlling change", and only 27 per cent holding that "most

managerial jobs require a person to compromise with his moral standards". The extent of doctrinal agreement makes one complaint about the courses—that there should be more expository teaching and less syndicate work—seem rather odd.

In this massive analysis I noticed two points that fitted in with my experience from outside, and seemed to me to need perhaps more attention than they receive in the book. One was that the job satisfaction of those in private industry was less after the course than before; whereas in the other occupations, the job satisfaction was much improved. Has private industry's ability to make the best use of people with training matched the amount of money which it has subscribed?

The other was that in their post-Henley jobs, people find the economic, financial and human relations problems the most frequent; while the most irksome by far (65 per cent) are those of human relations and problems in relations with higher authority. The latter is typical enough of the man coming back who wants to use the management techniques that he has acquired and finds no opportunity to do so; but to me a serious question is raised in management education generally, of whether enough weight is put on training in handling human relations problems. These are much more difficult questions to discuss than those of quantitative techniques and policy formulation; but this is unquestionably where the biggest problems of management lie, from the factory floor to the board room.

The experiment breaks interesting ground in classifying the managers into development patterns, distinguishing those who create change from those who build up their careers stage by stage and from those who do not enter easily into an organization but if handled properly can suddenly become so orientated. These too are well-known types—in the Treasury many years ago we used to call them the "carnivores" and the "herbivores" and the "marsupials"; and the report calls them "metamorphic", "incremental" and "tangential". There is an interesting question here, which the report begins to analyse, of which kind of training is best for each.

The real issue is whether, having distinguished into which category each promising man falls, the purpose should be to develop the strong characteristics or to seek to get more balanced men at the top by trying to reinforce their weaknesses. This is very difficult territory indeed; and it could well be

that the wise answer for career development in large-scale private industry is different from what is suitable for medium-scale private industry, and both different from the civil service.

I was left in some doubt at the end about whether the analysts had not perhaps sought to extract more information from the data than the data could be said to hold. Does a 3 month course play as decisive a role in a man's career as the analysis would imply? If so, there may be tens of thousands of people who should be on mid-career courses of this kind. It is difficult to see how the experiment could have been extended to provide a more definite answer, but I would like to know more of what the employers thought of the men when they came back from the course, and how the men performed in comparison with those who had not been to the course at all. As the quantity of management education expands, this is a problem of increasing relevance.

RICHARD CLARKE

## Power and Privilege

*Race and the Social Sciences.* Edited by Irwin Katz and Patricia Gurin. Pp. xii+387. (Basic Books: New York and London, 1969.) \$8.95.

THIS book comprises six lengthy essays by noted American scholars which emanated from papers and conversations at a conference in April 1967, held by the Center for Research on Conflict Resolution at the University of Michigan. In their preface, the editors state that the book is an effort to define the seminal research issues in American race relations today. They also note that "the gross inequalities of status, welfare, and opportunity that define Negro-white relations in America today urgently demand basic structural reforms in our society". But the essays do not delve deeply enough into the "seminal issues" and thus do not make the contribution, possible by social science, that is necessary to create "basic structural reform". All the essays are interesting, informative and provocative if for no other reason than that they highlight the gravity and immorality of race relations in America. If one is disposed to read between the lines and also has some awareness of the dimensions in race relations that have been omitted or under-emphasized (namely, inequality and institutionalized racism in American society), this volume seems but a moderate contribution to descriptive research, and a minor contribution to relevant analysis.



Herbert Hyman (Columbia University) wrote on social psychology, stressing socialization, attitude formation, perception and reference group identity, all critical to racial analysis; but as end points in research, freed from their political and economic contexts, they lead to sterile results. Hyman represented a dominant, traditional approach in American race research. Thomas Pettigrew (Harvard University) wrote on the Negro and education. His literature survey was exhaustive, special attention being paid to the famous Coleman report and the re-analysis of it by the Civil Rights Commission. In the effort to be "scientific", he applied standards of educational performance which may be outmoded in the evaluation of educational performance by minority group children. The principal emphasis was on integration and he seemed to assume some kind of linear integration model of academic performance with standards coming from the broader, white society.

Donald Matthews (University of North Carolina) developed criteria for a systems model (environmental inputs—policy making—outputs) that he thought would be useful for political science research. Though the systems model can be heuristic, Matthews may have over-used it; and this can yield a sterile exercise. He drew attention, however, to those political problems in race relations which others tended to pass over lightly. Karl Taeuber (University of Wisconsin) preferred a demographic approach and developed data on Negro fertility, family patterns, morbidity, mortality and population migration. His material is useful for information purposes, but he failed, as did most others, to place such data into the proper political-economic framework.

Charles Killingsworth (Michigan State University) wrote on "Jobs and Income for Negroes". He provided quite useful data on migration, occupational mobility, income, education, unemployment, under-employment, labour demand and supply. His analysis almost reached a point of vitality and relevance because he dealt with opportunity structures for disadvantaged persons, but his hints at solutions were conservative and uninspiring. James Coleman (Johns Hopkins University) developed a systems model as a basis for helping to explain "Race Relations and Social Change". He discussed power, deficits in Negro resources and the conversion of existing resources into usable resources that build efficacy and power. His theory was challenging and hit at important dimensions but one was left with the question: What is its social use?

The book is useful for data, information, ideas, a summary of mainstream social science and the "state of the art". It leaves many problems untackled

which would have been quickly developed by younger, radical analysts or representatives of the black community. Interestingly, most writers did not deal very directly with racism, but analysed the social system. Even then, they did not grapple very much with fundamental problems of political and economic power. Thus, they tended to reflect, once again, the difficulty "establishment" social scientists have in tackling the meat of relevant race relations analysis. ROBERT H. MAST

## Cell Mediated Immunity

*Cellular Immunology.* (An International Journal.) Edited by H. Sherwood Lawrence. Volume 1. (Academic: New York and London, May 1970.)

THE fault is with the system. The biomedical scientist of happier days could keep informed in his field (and related fields) by conscientiously reading the small number of papers written by a smaller number of fellow scientists published in a still smaller number of journals, annual reviews and so on. The system is now breaking down, and the response to this seems to be a further proliferation of publications which can only act to accelerate the breakdown process. This is one reaction to the appearance of *Cellular Immunology* but, in all fairness, my other reaction is that if another new journal must appear, it is justified in the field of cellular immunity.

There can be no disagreement about the rapid transformation of immunology into a discipline in its own right over the past decade, following more than half a century during which immunology as a hand-maiden of microbiology dealt primarily with resistance to infectious diseases. The immune response is now seen as the basis of a broad biological system for recognition which has important applications in many areas of basic science and in clinical medicine, including problems of transplantation, cancer (immunodiagnosis and possibly immunotherapy) immunopathology, development and genetics.

The form of the immune response which leads to the production of circulating antibodies has been the province of the well-developed subdiscipline of immunology known as immunochemistry. Immunochemists have been responsible for the rapid increase in knowledge of structure and function of the different classes of immunoglobulins (the circulating antibodies) and we seem near to the solution of the puzzle of the genetic control of the variable sequences of the amino-acids of peptide chains which must differ for each antibody specificity. It now seems, however, that resistance to many bacterial and virus diseases and perhaps parasitic diseases,

as well as important aspects of transplantation, cancer immunology and of immunopathology, depend on the kind of immune response which is not mediated by circulating antibodies. This area of "delayed type hypersensitivity" or "cellular immunity" includes, first, the specific cell-mediated immunity mediated by lymphocytes whose cell surfaces possess what must be immunoglobulin-like receptor sites to react with antigens and, second, the more non-specific cellular immunity mediated by macrophages (which may require activation by one of the products released following the reaction of the specifically sensitized lymphocytes with antigen).

In the first issue of *Cellular Immunology*, the editor-in-chief, H. Sherwood Lawrence, gives the rationale for the new journal, and the papers cover many aspects of the *in vitro* correlates of cell-mediated immunity which have made possible the recent rapid progress in this field. The reports that the lymphoid cells responsible for cell-mediated immunity differ in their morphological and physicochemical characteristics recall how the early concept that antibody was a single kind of globulin,  $\gamma$ -globulin, has been replaced by new knowledge of the heterogeneity of classes and subclasses of immunoglobulins, each with different structures and function. This issue also includes a report in experimental animals on the serum factors (presumably "blocking" antibodies) which have been shown to abrogate the inhibition caused by sensitized lymphocytes of the growth of tumour cells *in vitro*. Similarly, "blocking antibodies" have also been described in certain human cancers.

HOWARD C. GOODMAN

## Studies on Myelin

*Myelination.* By A. N. Davison and Alan Peters. With contributions by J. McC. Howell, J. E. Vaughn and L. I. Woolf. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xiv+238. (Thomas: Springfield, Illinois, August 1970.) \$13.50.

MYELIN is a structure which has received considerable attention from biologists, morphologists and biochemists in the thirty-five years since Schmitt investigated it with polarized light in 1936. Undoubtedly, it now has the added attraction to neurochemists that it can be obtained in bulk from the mammalian nervous system by relatively straightforward procedures, so that its chemical composition can be related to its physical properties and to its appearance under the electron microscope.

This rather expensive monograph was written largely by two prominent investigators in the field, and deals with the complex morphological and bio-

chemical events occurring during the formation of myelin in both the peripheral and central nervous system. The morphology of the process is lucidly considered at some length, and illustrated by some excellent photographs and diagrams. Peters has a good historical sense and invariably refers to such early pioneers in the field as Ranvier. Due note, too, is made of anomalies such as the apparent absence of the Schmidt-Lantermann clefts from myelin in the central nervous system.

Most of our knowledge of the biochemistry of myelin that has accumulated in the last decade is documented in the second chapter, and information is now available for a number of species. Both the composition and metabolism of the components are covered in some detail with an emphasis on that interesting characteristic of the myelin sheath, namely its considerable metabolic stability and negligible synthesis in adult life. It is apparent from this chapter, however, that there is still much to be learned about the metabolism of myelin proteins.

Another interesting feature of myelin is the fact that undernutrition at the "critical period" just before the period of rapid myelination causes a myelin deficit in the brain which can never be subsequently restored. Although studies on man have been few, and Davison is careful not to extrapolate the results of experiments on animals to the human species, the implication of these findings in areas of the world where infant malnutrition is commonplace may be of great importance.

In the postscript, the authors note that much remains to be learned about both the biochemical and morphological aspects of myelin pathology and this is apparent from the chapters on the subject. There is some scattered information on multiple sclerosis and some rarer human disorders; it is also of considerable interest that two human inborn errors of metabolism, phenylketonuria and maple-syrup urine disease (leucinos), are associated with a lack of myelin in the brain. There is much to be learned additionally from such mutants as the "quaking mouse" where the entire central (but not the peripheral) nervous system is deficient in myelin; no mention is made, however, of another mutant, the "jimpy mouse". Myelin is also deficient in many syndromes occurring in domestic animals, for example, swayback of sheep, which is associated with low levels of copper in their pastures. Some of these diseases are covered all too briefly, however, and chapter 5 becomes a list rather than an account.

I noted spelling mistakes on pp. 72, 116 and 144 and, in a column heading in Table 2—XVI, the word "mole" should read " $\mu$ mole". These are, never-

theless, trivial errors, and in many ways the book is attractive and lively.

G. B. ANSELL

## Traces of the Past

*Trace Fossils*. Edited by T. P. Crimes and J. C. Harper. (Proceedings of an International Conference held at Liverpool University in January 1970, under the auspices of the Liverpool Geological Society and sponsored by the International Union of Geological Sciences. *Geological Journal* special issue No. 3.) Pp. 547. (Seel House: Liverpool, December 1970.) £8.00; \$20.

"T. T. 'n B."—thus an eminent American geologist dismissed the various markings found on bedding surfaces recently. Tracks, trails and burrows were thus recognized as such but no interest was shown in them. This book, however, shows how wrong he was. *Trace Fossils* is a compendium of the papers presented at a conference on palaeo-ichnology held in Liverpool a year ago. The thirty-five papers from eighteen countries open up a new aspect of the interpretation of past environments which cannot be ignored by stratigraphers, sedimentologists or palaeogeographers.

Observations which emerge clearly from the conference are that careful analysis of the structures produced in sediments by the feeding, resting or locomotory activities of organisms shows that they can be related in many cases not only to the species or species group which made them, but also to the aspect of their life cycle which was involved, for example, young trilobites were swimmers and left no traces, while their elders crawled around making *Cruziana* trails or rested in *Rusophycus* hollows. Some traces, however, are not associated with body fossils (skeletal remains) and the makers of such traces are unknown. Analysis of these does permit some deduction of the method of formation, such as radial feeding from a vertical burrow, resulting in stellate trace fossils. Comparison with the tracks and burrows of present day molluscs, crustacea and annelids on shallow sea bottoms allows some interpretation of equivalent traces in sedimentary rocks even when no body fossil is known. Furthermore, study of the abundance and distribution of such present day traces shows how they can be related to water depth, sediment characteristics, energy levels of tides and currents and then to food supply. In turn the distribution and abundance of suitable traces in sedimentary rocks can provide evidence of the depth and direction of the shore line, which may not be available by other means. Because many such associations are found in rocks

otherwise devoid of fossils, a new form of evidence has become available for palaeogeographers. The traces may also indicate whether many "barren" sediments were deposited in fresh or salt water. Underwater photography of modern traces has solved some problems but provided others. Stellate markings at 3,000 m depth were made by unknown organisms; they also show that such trace fossils are unreliable as depth indicators.

Borings made by parasitic or commensal organisms in the shells of molluscs or other large invertebrates are not regarded by some as being trace fossils, but they are discussed in several contributions to this book; they are equally important as environment indicators. Micro-borings in shells can result from infestations by algae and fungi as well as bryozoans. Distinctions between these can be made by the scanning electron microscope and they are important, for an algal infestation would suggest photic depths, while fungal growth would not. One type of boring crossed by another may signify that the host shell has been transported between environments.

The contributions to this book cover all these topics and more. While some are largely descriptive, others emphasize the environmental significance. Some progress towards the use of trace fossils as indicators of stratigraphic age has been made, particularly in discriminating subtle differences in trilobites' *Cruziana* trails in Lower Palaeozoic rocks. The nomenclature of trace fossils is considered by many contributors: while realizing that the naming of traces is outside the rules of zoological nomenclature, most authors have adopted a Linnaean binomial system, and the simple fact of this almost universal practice seems to indicate that this is the only realistic way of dealing with the problem. Some 200 genera and species of trace fossil are discussed in the book, and some former differences of usage have been resolved.

The book is magnificently produced, remarkably free of misprints, well illustrated, and with comprehensive bibliographies at the end of each contribution. The editors are to be congratulated for producing it in under a year after the conference. Too expensive for a textbook, it will doubtless serve as a work of reference for many years. There are, however, some drawbacks and omissions. The contributions are arranged in alphabetical order of authors' names: why could they not have been grouped under topics? There are some differences of opinion expressed without comment from the editors. Could the editors not have arranged for a summary of findings in such groups? Topics omitted are, strangely, mostly connected with lime-



stones; is not *Stromatactis* a trace fossil, probably of plant origin? Indeed, plants on the whole are given short shrift in this compendium. Stromatolites are traces of the growth patterns of blue-green algae, themselves rarely preserved—but stromatolites are barely mentioned in this book. The numerous impressions of jellyfish(?), sea-pens(?) and so forth, in late Pre-Cambrian rocks, particularly at Ediacara in South Australia, are not mentioned, though several contributions make it clear that the chronological distribution of trace fossils shows that burrowing life forms appeared only in very late Pre-Cambrian times.

This book makes the science of palaeo-ichnology respectable, and it should be on every historical geologist's shelf. T. D. FORD

## Flow Mechanisms

*Annular Two-Phase Flow.* By G. F. Hewitt and N. S. Hall Taylor. Pp. ix + 310. (Pergamon: Oxford and New York, December 1970.) £7.50; \$20.

Now that the vast literature on two-phase flow in pipes seems to be past its peak, it is timely that an authoritative monograph on the subject should make its appearance, and what better stable could it come from than the Chemical Engineering Division of AERE, Harwell? Over the past 15 years Dr Hewitt and his co-workers have been amongst the leaders in this field, particularly in emphasizing the fundamental flow mechanisms involved in the flow of liquid-gas mixtures in vertical tubes and the implications these have for the heat transfer properties of such systems. This book is largely a consolidation of the published work of the authors and their co-workers, set in context with present knowledge by reference to nearly 400 published papers. As the title implies, it is concerned chiefly with the annular flow regime, and largely restricted to upward flow in vertical tubes. Reference to experimental results is usually confined to air-water or steam-water systems, as indeed is most of the literature in this field.

After discussing flow regimes, the authors proceed to apply momentum and energy balances to various flow models. The application to annular flow assuming a smooth interface is presented in detail. A chapter on empirical correlations concludes with an example of a pressure gradient calculation. Whether such a calculation will always yield the sort of agreement with experiment here demonstrated is an open question. The theory of interfacial waves is discussed in some detail and another chapter treats droplet entrainment very thoroughly.

Heat transfer, boiling and burnout

are dealt with in three of the twelve chapters. There is perhaps a tendency throughout to present theory for its own sake, exemplified in the discussion of the heat transfer resistance of an interface. If such a resistance is significant, it is certainly not in the situation illustrated in Fig. 10.1, where a considerable temperature gradient in the gas phase is shown. Nevertheless, these three chapters form an excellent and clearly presented summary of the present state of knowledge. The final chapter reports on a number of experimental techniques used in the Harwell laboratories and warns of pitfalls that await the inexperienced in this field. It should prove invaluable to the young research worker.

A comprehensive nomenclature list includes units of measurement, both British and metric. It is a pity that the units quoted are not all directly comparable with the SI unit conversion table, thoughtfully included for the uninitiated as an appendix. For some,  $\text{ft}^2/\text{s}^2$  are not the most obvious units for latent heat, though correct, unlike those quoted for thermal conductivity. But errors have been hard to find in this excellently produced book. The designer of two-phase systems may find few ready-made design techniques, but by studying this book he will certainly gain a better understanding of the beast he is up against. G. H. ANDERSON

## Tools for Research

*pH Meters.* By A. Wilson. (Laboratory Instrument and Techniques Series.) Pp. 119. (Kogan Page: London; Barnes and Noble: New York, 1970.) £2.00.

*Electron Microscopes.* By J. A. Swift. (Laboratory Instruments and Techniques Series.) Pp. 88. (Kogan Page: London; Barnes and Noble: New York, 1970.) £2.00.

*Gas Chromatography.* By C. Simpson. (Laboratory Instruments and Techniques Series.) Pp. 117. (Kogan Page: London; Barnes and Noble: New York, 1970.) £2.50.

THESE three titles announce the beginning of a series of books about instruments and techniques in common use in research laboratories. The level of readership at which the books are aimed is a variable because each book can be subdivided and is not likely to be read in its entirety by all readers. A section at the end of each book is devoted to tables which give a broad picture of the commercial instruments available together with their individual characteristics and range of functions. The books would therefore seem to be suitable both for people involved in the purchase of instruments such as electron microscopes and chromatographs, and

for the student who will presumably use the body of the text to obtain an account of the theory and practice of the particular technique.

The book on electron microscopes is rather spoilt by a mistake on page 12 at the end of a section in which the author describes advantages of an electron beam over a light beam; during the calculation of the wavelength of electrons accelerated by a potential difference  $V$ , reference is made back to the wrong equation and the expression for  $\lambda$  (metres) needs to be multiplied by  $10^{-10} V^{1/2}$ . The calculation to establish a sample wavelength is, however, correct and the point is validly made. The remainder of the book is remarkable for its account of the many different uses to which a commercial electron microscope can be put (for example, X-ray microanalysis and magnetic domain study). The basic functioning of an electron microscope and its attachments are well described although one might have hoped for a clearer diagram of a complete microscope than the longitudinal section of the commercial model illustrated. In this book, as in the other two, the general appearance leaves much to be desired and there is a complete lack of justification of the right text margin.

The first page of *Gas Chromatography* might be a little daunting for the reader approaching the topic for the first time because it seems to assume an understanding of precisely why different solutes should emerge from the column at different times. The quality of the printing on several pages is bad and part of one page is completely unreadable; figure 3.3 does not seem to be referred to in the text at all. In a few cases it would have been better if the order of presentation had been somewhat different—for example, several of the solute detectors are referred to in a way which demands some appreciation of their mode of operation before they were fully described. Such a list of criticisms should not, however, detract from the value of the book as a very comprehensive guide to the art of gas chromatography which will answer or suggest a source for the answer to most questions which are likely to arise.

The book *pH Meters* is also remarkable for its comprehensive coverage of the topic and, although the abundance of mathematics at the beginning of the book could deter the casual reader, anyone who wants information about methods of measuring pH, including a brief description of colorimetric indicators, should be able to find it. The book not only considers the measurement of pH in a chemistry laboratory but also discusses pH measurement and control in industry and the measurement of pH in medical and biological laboratories.

ROGER WOODHAM

# CORRESPONDENCE

## Credit for Authorship

SIR,—Your correspondent (*Nature*, **229**, 436; 1971) on multiple authorship seems to wish to perpetuate the axiom "publish or perish" by his suggestion that each author be allocated a fractional amount of credit for a paper of which he is co-author. Whilst I recognize that this has become part of the "rat race", I abhor someone's trying to promote it. It is beyond my comprehension how one would arrive at the contribution of a particular author to a paper—for example, 10% practical contribution, 5% theoretical contribution and 55% "big name" contribution.

Perhaps, following on the lines of Schmid's letter, we should award a star rating to articles in a specific publication ("—and the five star paper of the month is") and conduct surveys on the lines of the one by Panton and Reuben<sup>1</sup> to award a star rating to each scientific journal enabling authors to boast and ask their friends "How many stars do you have this year?". Or possibly one should take the Comprehensive Rating of Academic Proficiency formula proposed by Ramaley *et al.*<sup>2</sup> if one is concerned with promotion and "success". The point here, of course, is that one doesn't start with the author, one starts with the quality of the scientific paper.

Surely the publication of a scientist's work is for the advancement of science and the sharing of one's knowledge and findings with the scientists of the world—or am I being too naive?

Yours faithfully,

FRANK SNAPE

Department of Chemistry,  
Dalhousie University,  
Halifax, Nova Scotia

<sup>1</sup> Panton, D., and Reuben, B. G., *Chem. in Britain*, **7**, 18 (1971).

<sup>2</sup> Ramaley, L., Coston, G., and Butcher, J., *AAUP Bulletin*, **55**, 279 (1969).

## Expensive Meat

SIR,—The non-biologist (*Nature*, **229**, 435; 1971) suggested that tissue culture techniques be considered as a future supply of "meat". We have suggested that it may be possible to provide blood and blood products by the growth of human haematopoietic cells<sup>1</sup> and considered the suggestion as quite "wild". The growth of cells for meat is several logs more remote even though the scientific capability is available. The limitations are (1) the division rate of cells acceptable for food; (2) the cost of the culture media; (3) expansion of the kind of "cell plants" that we at present use for growing large amounts of

human cells<sup>2</sup>; and (4) avoidance of the spread of dangerous infections and abnormal genetic material and the like.

Normal human cells have a maximum division rate of approximately 19 to 20 hours and would, therefore, be unsuitable from a practical standpoint but one could feast on one's own lymphocytes if limited to hors d'oeuvres. We could grow about 1 kg of human lymphocytes per day in our 1,200 l. culture unit. The cost per kg of this "meat" with present techniques would be as little as \$2,500 for the culture media.

The maximal division rate of mouse cells is about 9.5 h and would still pose a severe limitation on production even if a growth rate of 2 g of cells per kg of media could be sustained—and mouse meat may not be tasty.

We have fed residual cultured human cells to tropical fish for several years and can testify that the diet was apparently nutritious, supported rapid reproduction, and was not associated with the development of tumours.

Even the ethical problem will not be solved once we learn to grow animals and humans from single cells.

Yours faithfully,

GEORGE E. MOORE

Roswell Park Memorial Institute,  
666 Elm Street,  
Buffalo,  
New York 14203

<sup>1</sup> Moore, G. E., and Glick, J. L., *Surgical Clinics of North America*, **47**, 1315 (1967).

<sup>2</sup> Moore, G. E., and Vosseller, G. V., in *Methods in Enzymology* (edit. by Jakoby, W. B.) (in the press).

## Numerical Etymology

SIR,—The suggestion of R. M. Burroughs (*Nature*, **229**, 142; 1971) for the consistent use of the sequence "million, billion, trillion, . . ." for the numbers  $10^6$ , agreeing with standard usage in most of the world, and of another sequence "milliard, billiard, trilliard, . . ." for the numbers  $10^{6(n+1)}$ , formed analogously from the once universal "milliard", is an eminently sensible and natural one. It is not a new suggestion, however; the consistent use of both sequences was, I believe, first proposed by L. Gustave Du Pasquier (*Proc. Intern. Math. Congr.*, **2**, 975, University of Toronto Press, 1928).

The difficulty with N. W. Pirie's proposal of "gillion" and "tillion" (*Nature*, **229**, 283; 1971) is—where do we go after "giant" and "monster" (Giga < γίγας = giant, Tera < τέρας = monster)?

"King-size", perhaps? Unfortunately, βασιλεύς would give "billion" again—now  $10^{15}$ . I do not understand Pirie's criticism of "milliard" as a "vague word". I have never seen "milliard" defined or used to mean anything except  $10^9$ .

Returning to the Du Pasquier-Burroughs suggestion, a minor criticism is that, like the SI prefixes, it perpetuates the sextal (and ternary) system for counting indices, while the rest of our number system is decimal. In mathematical work where large integers, for example large prime numbers, have to be written out at length, and also in tables of more than 10 decimal places, it is not unusual for the digits to be arranged in blocks of five, not of six or three. Could some thought not be given to number names (and SI prefixes) based on powers of  $10^{10}$ ? Individual names are already available in English for all the powers of 10 with single digit exponents, except for  $10^8$ ; we already have "ten", "hundred", "thousand", "million", and "milliard", and the best dictionaries still list the borrowings "myriad" ( $10^4$ ), "lac" or "lakh" ( $10^5$ ), and "crore" ( $10^7$ ). All we need do is invent a name for  $10^8$  and some systematic terminology for  $10^{10}$ ,  $10^{20}$ , . . . ,  $10^{80}$ . For  $10^{100}$ , we have, of course, Kasner's "googol".

Yours faithfully,

THOMAS WRAY

Department of Energy,  
Mines and Resources,  
Ottawa 4,  
Ontario

## British Diary

### Monday, March 15

**Low Cost Digital Voltmeters** (2.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

**Mitochondrial Structure and Function** (5 p.m.) Professor Lars Ernster, University of London, in the Chemistry Auditorium, University College London, Gower Street, London WC1. (Further lectures on March 17 and 19.)

**Physicochemical Aspects of Herbicide Selectivity** (1.45 p.m. symposium) Society of Chemical Industry, Pesticides Group, at 14 Belgrave Square, London SW1.

**The 300 GeV Accelerator: For and Against** (8 p.m. debate) British Society for Social Responsibility in Science, at the Institute of Contemporary Arts, Nash House, The Mall, London SW1.



**Water and Industry** (6 p.m.) Dr C. J. Jackson, Royal Society of Arts, at John Adam Street, Adelphi, London WC2. (First of four Cantor Lectures on "Problems of Pollution".)

## Tuesday, March 16

**Astronomy in the Eighteenth and Nineteenth Centuries** (5.15 p.m.) Dr G. J. Whitrow, University of London, at Bedford College, Regent's Park, London NW1.

**Computers in Medicine** (two-day symposium) World Organization of General Systems and Cybernetics, British Chapter, at Blackburn College of Technology and Design, Blackburn.

**Electrical Safety in Hazardous Environments** (three-day conference) Institution of Electrical Engineers, in association with the Association of Mining and Mechanical Engineers, the Institution of Electronic and Radio Engineers, the Institute of Measurement and Control, the Institution of Mechanical Engineers, and the Institution of Mining Engineers, at the Institution of Electrical Engineers, Savoy Place, London WC2.

**Environmental Testing of Advanced Gas Cooled Reactor Components** (6.30 p.m.) Society of Environmental Engineers, at the University, Salford.

**Methanol** (6 p.m.) Mr I. McLeod and Mr D. Hanson, Society of Chemical Industry, London Section, at 14 Belgrave Square, London SW1.

**New Metals and Alloys** (7.45 p.m.) Mr J. R. B. Gilbert, Leeds Metallurgical Society, at the Houldsworth School of Applied Science, The University, Leeds.

**Nitrogen Fertilizers and Crop Responses** (10 a.m.) Society of Chemical Industry, Agriculture Group, jointly with the Fertilizer Society, at 14 Belgrave Square, London SW1.

**Sir Hans Sloane and his Natural History Collections** (6.30 p.m.) Mr Roy Porter, British Museum (Natural History) and the Victorian Society, in the Lecture Hall, British Museum (Natural History), Cromwell Road, London SW7.

**The Study of Surfaces Using Interferometry** (5.30 p.m.) Professor S. Tolansky, FRS, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Sixth Form Pupils from Schools in London and the Home Counties. To be repeated on March 17, 23 and 24.)

## Wednesday, March 17

**Applications of the Scanning Electron Microscope** (7 p.m.) Mr H. Wells, Oil and Colour Chemists' Association, at the Borough Polytechnic, Borough Road, London SE1.

**Data Communications—Studies for a Public Service** (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

**Data Logging Techniques** (6 p.m.) Mr J. T. Kennair, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

**The Mathematical Analysis of Mill Circuits** (5.30 p.m.) Professor H. E. Rose, University of London, at King's College, Strand, London WC2. (Third of four lectures on "An Introduction to Comminution Technology".)

## Thursday, March 18

**Additives for Paint and Printing Inks** (7 p.m.) Mr R. G. Kinsman, Oil and Colour Chemists' Association, at the Beech Tree Hotel, Beaconsfield, Bucks.

**"Honest Jack" Fuller** (5.30 p.m.) Mr James Lawrie, Royal Institution, Library Circle, at 21 Albemarle Street, London W1.

**Microelectronics** (6.30 p.m.) Mr E. T. Emms, Institution of Electrical Engineers, London Graduate and Students Section, at Savoy Place, London WC2.

**Technical Innovation in Illuminating Engineering** (5.30 p.m.) Mr J. R. Coaton, Institution of Electrical Engineers, at Savoy Place, London WC2.

**The Activities of the Non-Destructive Testing Centre at Harwell** (7.30 p.m.) Mr R. S. Sharpe, East Midlands Metallurgical Society, in the Lecture Theatre, Edward Herbert Building,

Loughborough University, Loughborough.

**The Properties of Bitumen in Relation to Road Performance—their Measurement, Interpretation and Specification** (5.50 p.m.) Mr G. M. Dormon and Mr H. M. Snashall, Society of Chemical Industry, Road and Building Materials Group, at 14 Belgrave Square, London SW1.

## Friday, March 19

**Computer/Patient Relationships** (2 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

**Determination of Common Anions** (6.30 p.m. discussion meeting) Society for Analytical Chemistry, at the George Hotel, Chepstow.

**Forensic Science** (6.30 p.m.) Mr P. J. Cobb, Oil and Colour Chemists' Association, at the Chamber of Commerce House, 75 Harborne Road, Birmingham 15.

**Recent Developments in Organic Electrochemistry** (2.30 p.m.) Mr M. E. Peover and Mr D. Pletcher, Society for Analytical Chemistry, Electroanalytical Group, at the College of Further Education, Handbridge, Chester.

**The Effects of Newer Types of Insulant on Portable Tools** (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

## Monday, March 22

**Air Pollution** (6 p.m.) Dr A. J. Robinson, Royal Society of Arts, at John Adam Street, London WC2. (Second of four Cantor Lectures on "Pollution".)

**Artificial and Natural Pollution Tests on Outdoor 400 kV Substation Insulators** (5.30 p.m.) Mr C. H. A. Ely, Mr R. G. Kingston and Mr P. J. Lambeth, Institution of Electrical Engineers, at Savoy Place, London WC2.

**Circuit Theory** (vacation school, twelve days) Institution of Electrical Engineers, at the University College of North Wales, Bangor, North Wales.

**Ferrite Microstrips** (10.30 a.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

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